Has Psychology Ignored Our Gut Feelings? Exploring the Relationship Between Gut Microbiota and Psychological Symptoms: A Call for a Paradigm Shift.

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Abstract

The profession of psychology has long been entrenched in a traditional central nervous system (CNS) centric framework. This specialisation has had its benefits and contributed to current knowledge of psychological symptoms and disorders. However, this reductionist approach has led to gaps in knowledge that will continue to persist without a broader appreciation of the complexity of the human body. Broader consideration of bodily systems may provide greater insight into the aetiology, diagnosis, treatment, and prevention of psychological symptoms and disorders.

The enteric nervous system (ENS) and its resident gut microbiota (GM) has emerged as a peripheral influence on psychological functioning. The GM refers to the trillions of microorganisms residing in the gut including, but not limited to, bacteria, fungi, and protozoa. The GM has coevolved with its human hosts to share a highly complex multidirectional relationship. In a state of symbiosis, GM play a key role in protecting against pathogen colonisation, strengthening and maintaining the epithelial barrier, and nutrient absorption through metabolism, therefore promoting host health. On the other hand, in a state of dysbiosis (imbalances in the composition of GM), this mutually beneficial relationship between host and GM shifts towards a more antagonistic one. Dysbiosis of GM, as well as specific gut microbes themselves, have been associated with a wide range of psychological symptoms and disorders. To date, of the organisms that reside within the GM, bacteria have received the majority of attention in brain-gut-microbiota axis (BGMA) research.

This thesis broadly aims to position the BGMA as falling within the purview of psychologists, while also exploring the concept of the microgenderome in a series of three papers. Paper 1 is a review paper which aimed to demonstrate that GM are intrinsically linked with each stage of psychological disorder, from aetiology through to treatment and prevention. The paper was framed around the Four P model of case formulation, often used in psychological practice.

With the neglect of focus on other microorganisms, paper 2 was the first to investigate the effect of these protozoa on psychological symptom severity. Specifically, Paper 2 presents the results of a cross-sectional, retrospective study of the differences in Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue symptom severity between individuals negative for intestinal protozoa (*n*= 563) compared to those positive for common protozoa *Blastocystis* sp. (*n*= 274), *Dientamoeba fragilis* (*n*= 69), or both (*n*= 73). The findings demonstrated that there was no statistically significant effect of either protozoan, or co-carriage, on psychological symptom severity for either males or females.

Utilising correlational analyses, a retrospective cross-sectional exploration of the association between GM and Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue symptom severity was carried out in Paper 3. While the overall sample was made up of 4610 clinically diverse participants, sample size for each correlational analysis was dependent on available data. The pattern of associations between several GM species and psychological symptom severity were distinctly different between males and females, providing support for the microgenderome. The results demonstrated that some bacterial species found in common probiotic supplements were positively correlated with symptom severity. The results provide support for the notion that, in future, modulation of GM may be appropriate as an ancillary treatment of psychological symptoms, however further research is needed before their implementation in treatment plans.

Collectively, this thesis demonstrates that expanding the CNS-centric approach to include peripheral systems may revolutionise the way that psychological illness, and its prevention and treatment are conceptualised. Future directions for research and clinical practice are discussed which include methodological and practical challenges that must be overcome to substantiate the need for a paradigm shift for the discipline of psychology.

Student Declaration

"I, Michael Ganci, declare that the PhD thesis entitled **Has Psychology Ignored Our Gut Feelings? Exploring the Relationship Between Gut Microbiota and Psychological Symptoms: A Call for a Paradigm Shift** is no more than 80,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

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"All research procedures reported in the thesis were approved by the Victoria University Human Research Ethics Committee; HRE 16-071."

I declare that I have received a scholarship to complete this PhD with financial support from an industry partner, Bioscreen and Victoria University. This was an untied contribution from Bioscreen administered through Victoria University, with no restrictions on publication.

Signature: Michael Ganci  Date: 08/11/2021

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Contact was unable to be made with two co-authors listed in the documentation below (Dr. Henry Butt and Dr. Jean Tyrrell), and as such their signatures were unable to be secured.

The supervisors of this thesis have signed below, attesting to the fact that the declarations in relation to the contribution of all authors for each paper is accurate.

A/Prof. Michelle Ball

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Title of Paper/Journal/Book: The role of the brain-gut-microbiota axis (BGMA) in psychology: the importance of considering gut microbiota in the development, perpetuation, and treatment of psychological disorders.

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Emra Suleyman	5	concept, input into manuscript	[redacted]	10/09/21
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List of Abbreviations

16S rRNA	ribosomal ribonucleic acid
ANS	autonomic nervous system
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BGMA	brain-gut-microbiota axis
BPQ	Bioscreen patient questionnaire
CFQ	cognitive failures questionnaire
CFU	colony forming unit
CFU/g	colony forming unit per gram of stool
CNS	central nervous system
CRF	corticotropin-releasing factor
DASS-21	depression, anxiety, and stress scales (21 item version)
DNA	deoxyribonucleic acid
EEA	environment of evolutionary adaptiveness
EFA	exploratory factor analysis
ENS	enteric nervous system
FMA	faecal microbial analysis
FMT	faecal microbial transplant
GABA	gamma aminobutyric acid
GF	germ free
GI	gastrointestinal
GM	gut microbiota
HPA axis	hypothalamic pituitary adrenal axis
IgG	immunoglobin-G
LCA	latent class analysis
MALDI-TOF	microflex matrix assisted laser desorption ionization-time of flight
MANOVA	multivariate analysis of variance
MCAR	missing completely at random
ME/CFS	myalgic encephalomyelitis/chronic fatigue syndrome
ML	maximum likelihood

OECD	organisation for economic cooperation and development
PCR	polymerase chain reaction
PSQI	Pittsburgh sleep quality index
rRNA	ribosomal ribonucleic acid
SCFAs	short-chain fatty acids
SEM	structural equation modelling
SIBO	small intestinal bacterial overgrowth
SPF	specific pathogen free
WHO	world health organisation

Chapter 1: Contextualising the Problem

Over the last decade or so, research investigating the brain-gut-microbiota axis (BGMA) has proliferated. Briefly, the BGMA refers to the multidirectional relationship between the brain and the gut, and more specifically, the gut microbiota (GM); the ecosystem of microorganisms that inhabit the gut. The role of GM in human health and disease has been well established (Clemente et al., 2012; Marchesi et al., 2016; Sekirov et al., 2010). Specifically, there is a growing body of literature that has found associations between GM and psychological symptoms and disorders (e.g., Clapp et al., 2017; Dinan & Cryan, 2017; Foster et al., 2017). The relationship between the gut and disease is by no means a new concept. Hippocrates, considered to be the father of modern medicine, is often quoted as stating that 'all disease begins in the gut' approximately 2000 years ago. Additionally, traditional Chinese medicine has, and continues to, focus heavily on natural treatments which act through the function of the gut and its resident microbiota (Li et al., 2009). However, in Western medicine the gut has only relatively recently been considered as a potential focal point for explaining and treating disease (Bischoff, 2011; Clapp et al., 2017). As a result, conceptualisations of wider systemic disorder arising from the gut has given rise to questions regarding whether a paradigm shift within certain specialisations, such as psychology, is needed (Allen et al., 2017). Cartesian dualism has undoubtedly played a role in the reductionist and specialised way in which disease is conceptualised, diagnosed, and treated in the modern world. Modern medical practice has necessarily high standards of evidence, and change comes slowly. Nonetheless, there are early signs of a shift towards more holistic and personalised conceptualisations of disease.

Emerging evidence linking some psychological symptoms with gut health implores modern psychology to consider the gut as a possible target of intervention. Despite this, the discipline of psychology remains predominantly central nervous system (CNS)-centric. This tendency to continue to operate within a CNS-centric framework is likely the result of applying discipline specific knowledge gained through many years of education and clinical experience, and rightly so. A lack of exposure to information relating to the BGMA precludes the ability to incorporate it into psychological practice. BGMA research is necessarily multidisciplinary, however it is often presented in a way that makes it less accessible to psychologists. This is a result of the discussion of GM unavoidably using language that psychologists may be less familiar with. For example, the different types of microorganisms, differences in taxonomic ranks, microbiological methods of analysis, and terms related to the quantification of GM. The current thesis is written specifically with a psychology audience in mind, and attempts to bring clarity to these somewhat foreign concepts. With the goal of making BGMA research more accessible to a psychology audience, Paper 1 presents a review of the literature regarding the associations between GM and psychological disorders in the context of

the Four P model of case formulation. In doing so, the paper demonstrates how GM are related to each stage of the disease process, from factors which may predispose an individual to psychological disorders, as well as factors that may precipitate and perpetuate these disorders. The paper also demonstrates the potential protective nature of GM for healthy functioning. Paper 1 provides a psychological context for the two original research studies presented within this thesis (Paper 2 and Paper 3).

Following Paper 1, an expanded review of the literature is presented which further contextualises the GM and their relationship with psychological symptoms. Concepts that are discussed in Paper 1 are further elaborated such as the pressures which have driven co-evolution between humans and their resident GM. Additionally, the concept of the microgenderome is discussed, which proposes that sex hormones modulate GM resulting in sex-specific host-microbiota interactions (Clarke et al., 2013; Mulak et al., 2014; Vemuri et al., 2019). The potential mechanisms through which GM may exert their function on psychological symptom expression is also discussed. This chapter concludes by identifying a gap in the literature whereby the majority of BGMA research focuses solely on the bacterial component of the GM. Given that the GM is a complex ecosystem which is also comprised of protozoa, fungi, archaea, and viruses, this presents a clear need for further research.

Following this expanded literature review, the general method will be presented which outlines the original plan for this thesis, and discusses challenges relating to the retrospective cross-sectional dataset used for statistical evaluations associated with this project, particularly the issue of missing data. The challenges faced in the current study reflect those of microbiota data more generally. However, in reviewing the BGMA literature, discussion regarding how missing data is dealt with is absent. This section details the process undertaken to ensure that the analyses conducted in the papers presented within this thesis are appropriate and would lead to results and conclusions that are valid and meaningful. Given the retrospective nature of the dataset used in this thesis, psychological symptom severity was assessed using the Bioscreen Patient Questionnaire (BPQ). An exploratory factor analysis (EFA) is used to mathematically derive symptom domains to be used in Paper 2 and Paper 3. (The results of this factor analysis are presented in online resource 1 for Paper 2).

Paper 2 follows. In taking the first step to fill a gap in the literature, Paper 2 is the first study to investigate the effect of two common intestinal protozoa (*Blastocystis* and *Dientamoeba fragilis*) on self-reported psychological symptom severity across the four domains considered in this thesis. To date, research investigating their effect on human health has focused primarily on

gastrointestinal symptoms. Currently, health outcomes associated with these two protozoa are disparate and controversial (e.g., Garcia, 2016; Lepczyńska et al., 2017). This controversy extends to whether there is a need to treat *Blastocystis* or *D. fragilis*, with such treatments potentially having an impact on the gut ecosystem more broadly (Weir & Le, 2020). These inconsistencies and controversy necessitate further research. Paper 2 also explores whether the effect of *Blastocystis* and/or *D. fragilis* differs as a function of sex, exploring the microgenderome in the context of intestinal protozoa. This study is an important first step as the GM is a complex ecosystem which is not just comprised of bacteria, and therefore attention must be given to the other members of this community, such as protozoa. More attention on the non-bacterial members of this ecosystem will improve the overall understanding of the host-microbiota relationship. While addressing this important gap in the literature, this paper also informs the inclusion/exclusion criteria for Paper 3. Due to the inconsistencies in previous research and controversy regarding the role of these two protozoa in human health, Paper 2 is a necessary first step to determine whether psychological symptom expression is influenced by underlying protozoan carriage.

Paper 3 explores the association between bacterial and fungal microbiota species and psychological symptom expression. Viruses and archaea, which are also constituents of the GM, were not included in the analyses of Paper 3 as they were not measured in the retrospectively collected dataset. This paper will contribute to areas of research which have received relatively less attention in the literature. First, Paper 3 will focus on bacteria and fungi at the species level, where the majority of BGMA research has been conducted at higher taxonomic ranks (e.g., genus, family, or phylum). To explain briefly, higher taxonomic ranks (such as phylum) are broader and organisms within them are more genetically diverse, while lower taxonomic ranks (such as species) are more genetically similar (Al Bander et al., 2020). Due to the heterogeneity found within the higher taxonomic ranks, it is necessary to explore these associations at the species level. For example, the genus *Clostridium* has species within it that are considered pathogenic (e.g., *C. difficile*; Guo et al. 2020) while other Clostridia species are considered commensal, or even beneficial (Lopetuso et al., 2013; Sun et al., 2018). As such, investigation at the genus level (or at higher taxonomic ranks) misses these important nuances. Second, Paper 3 also explores the associations between psychological symptom severity and fungal species. While there is a body of literature that has investigated the associations between psychological symptom expression and fungi, this area of research continues to lag behind that which investigates bacteria exclusively. It is important to explore the associations between fungi and psychological symptom expression as previous research has demonstrated some links to exist (e.g., Rucklidge, 2013; Severance et al., 2016). Finally, paper 3 also explores the microgenderome in the context of bacterial and fungal species. Again, this allows

for exploration of the nuances of species level associations to be even more specific and detailed. A growing body of research demonstrates that associations between GM and psychological symptom expression are sex specific. Paper 3 extends on research conducted by Wallis et al. (2016) who determined that despite similar GM composition between males and females, certain genera were positively associated with symptoms in one sex, while negatively associated with symptoms in the opposite sex.

The final chapter will provide a synthesis of the main research findings of the papers presented within this thesis. It will also include a critical review which highlights the parallels between psychological and microbiological research. It will discuss the importance of considering the GM as an ecosystem from a more holistic perspective. Furthermore, the importance of taking a holistic and multidisciplinary approach to the conceptualisation of psychological disorders will also be discussed. It is proposed that by reconceptualising psychological disorders from a more holistic and multidisciplinary perspective, the discipline of psychology is primed for a shift away from a CNS-centric approach to diagnosis and treatment. The clinical relevance of this is that modulation of GM may present as a viable auxiliary treatment option. As such, it is argued that consideration of the GM will lead to a greater understanding of the aetiology of a client's presenting problem and will improve treatment efficacy. This shift towards a more holistic and multidisciplinary approach to psychology is in line with a recent push for personalised medicine. This chapter ends with a philosophical discussion regarding the reconceptualization of not only psychology, but of what it is to be human. Specifically, the concept of the holobiont will be discussed along with the implications of this new way of thinking.

Chapter 2: The role of the brain-gut-microbiota axis in psychology: The importance of considering gut microbiota in the development, perpetuation, and treatment of psychological disorders

Paper 1 presents a review of the literature regarding the associations between GM and psychological symptom expression from the perspective of the Four P model of case formulation. This paper provides the context and rationale for the two original research papers presented within this thesis.

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REVIEW

Brain and Behavior Open Access WILEY

The role of the brain–gut–microbiota axis in psychology: The importance of considering gut microbiota in the development, perpetuation, and treatment of psychological disorders

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Abstract

Introduction: The prevalence of psychological disorders remains stable despite steady increases in pharmacological treatments suggesting the need for auxiliary treatment options. Consideration of the brain–gut–microbiota axis (BGMA) has made inroads into reconceptualizing psychological illness from a more holistic perspective. While our understanding of the precise role of gut microbiota (GM) in psychological illness is in its infancy, it represents an attractive target for novel interventions.

Method: An extensive review of relevant literature was undertaken.

Results: Gut microbiota are proposed to directly and indirectly influence mood, cognition, and behavior which are key components of mental health. This paper outlines how GM may be implicated in psychological disorders from etiology through to treatment and prevention using the Four P model of case formulation.

Conclusion: Moving forward, integration of GM into the conceptualization and treatment of psychological illness will require the discipline of psychology to undergo a significant paradigm shift. While the importance of the GM in psychological well-being must be respected, it is not proposed to be a panacea, but instead, an additional arm to a multidisciplinary approach to treatment and prevention.

KEYWORDS

allostatic load, gut microbiota, precipitating factors, predisposing factors, protective factors, psychology

1 | INTRODUCTION

Burgeoning research regarding the role of the gut and its microbial inhabitants in the pathophysiology of psychological illness is gaining momentum. Early evidence points to gut microbiota (GM) as a possible missing link in the conceptualization and treatment of psychological illness that sees disparities between conventional treatment methods and prevalence rates. When discussing the role of GM in behavior, health, and disease, it is important to pay respect to the intertwined coevolution between humans and our resident microbes. It is suggested that the sharp increase in various disease states over the last 50–100 years (Campell, 2014; Linneberg et al., 2000) can be, at least in part, explained by relatively recent dietary and lifestyle changes in the context of human evolution (Broussard & Devkota, 2016). Currently, humans, particularly those in industrialized countries, are living in an environment to which they have not adaptively evolved (Gluckman, Low, Buklijas, Hanson, & Beedle, 2011). An unintended consequence of industrialization, these

changes are putting the GM under evolutionary pressure to shift from a previously mutualistic relationship with their human host to a more antagonistic one (Broussard & Devkota, 2016; Quercia et al., 2014). This is due to human evolution requiring significantly more time (Uyeda, Hansen, Arnold, & Pienaar, 2011) compared to single-celled organisms such as GM that evolve and adapt to environmental and internal states much more rapidly (within as little as 24 hr; David et al., 2014; Wu et al., 2011). These variations in the evolutionary pressures and capabilities of the two components (host and GM) of a single ecosystem (the holobiont; Theis et al., 2016) result in systemic disharmony.

This systemic disharmony leads to symptomatology and disease states that are the primary target for intervention in current medical models, which precludes effective etiology-focused prevention (Marvasti & Stafford, 2012). This is exacerbated given that current medical models are heavily skewed toward treatment over prevention (Singh, 2010). While there is disagreement over whether the occurrence of common mental disorders are increasing or whether their prevalence remains consistent (Friedrich, 2017; Harvey et al., 2017), the consumption of antidepressant drugs doubled in most, if not all, Organisation for Economic Co-operation and Development (OECD) countries between 2000 and 2015 (OECD, 2017). Despite this, depression has recently taken over as the leading cause of disability worldwide with anxiety also in the top 10 leading causes of disability (World Health Organisation (WHO) 2017). Furthermore, subclinical psychological symptoms that do not meet the full Diagnostic and Statistical Manual for Mental Disorders (DSM) or International Classification of Diseases (ICD) diagnostic criteria are also prevalent (Angst, Merikangas, & Preisig, 1997; Haller, Cramer, Lauche, Gass, & Dobos, 2014; Mathieson, Collings, & Dowell, 2009). These subclinical symptoms lead to impairment in psychosocial and work functioning, are a major determinant of sick leave, contribute to the use of psychotropic drugs and primary health care services, and reduce quality of life (Haller et al., 2014; Johansen & Dittrich, 2013; Mendlowicz & Stein, 2000).

The conceptualization of the human body as being made up of separate and distinguishable systems is likely to have contributed to our current poor appreciation of the complexity of mechanisms that underlie the etiology and progression of disease. Psychology, just like many other healthcare professions and medical science disciplines, specialize and focus on specific body systems. For example, treatment of psychological symptoms and disorders tends to be central nervous system (CNS)-centric with the brain being the principle target for both psychotherapy and psychopharmacology while peripheral systems, such as the gut, receive little attention. While there is no doubt that specialization has its benefits and has contributed to the progression of medical knowledge, it also has its drawbacks. Operating within these constrictive distinctions imposed by disciplinary specialization means that the complex interplay between various body systems essential to proper functioning is overlooked. The true etiology of disease is likely to come from dysregulations in this interplay which may also go some way to explaining high levels of comorbidity, particularly between functional gastrointestinal disorders such as IBS and psychological conditions (Garakani et al., 2003; Lee et al., 2015).

Within the field of psychology, an integral part of intervention is case formulation. Essentially, case formulation involves the synthesis of information about a patient (typically gained through clinical interviews and formalized cognitive testing) in a meaningful way to facilitate the development of a treatment plan. A commonly used model in structuring a case formulation is that of the Four Ps which represent the predisposing, precipitating, perpetuating, and protective factors related to a client's presenting problem and thus the targets of psychological intervention. As information is drawn from clinical interviews and cognitive testing, the functioning of a client's gut is seldom considered in their formulation; thus, important diagnostic and treatment options may be missed. It is the contention of this paper to demonstrate that GM are intimately linked with each of these four pillars of psychological intervention and thus each stage of disease, from etiology through to treatment and prevention. This paper adds to the burgeoning research into the brain–gut–microbiota axis (BGMA; Kelly, Clarke, Cryan, & Dinan, 2016) demonstrating that the discipline of psychology is on the cusp of a significant paradigm shift, moving away from CNS-centric approaches toward a more holistic conceptualization of health and disease which integrates other body systems. We echo the sentiment of Allen, Dinan, Clarke, and Cryan (2017) who call for a challenge to the reductionist approaches in psychology in favor of a multidisciplinary approach to conceptualizing and treating psychological disorders. In taking the unique approach of the Four P model of case formulation, this paper intends to review existing research on associations between GM and psychological outcomes, compiling it in a way that is more accessible to psychologists, especially those who have little previous knowledge regarding the BGMA.

1.1 | The brain–gut–microbiota axis

The BGMA is increasingly being recognized as playing an important role in homeostasis and consequentially, health and disease states (e.g., Mu, Yang, & Zhu, 2016; Rea, Dinan, & Cryan, 2016). The BGMA refers to the relationship between the brain and the gut while acknowledging the important moderating role of GM (e.g., Carabotti, Scirocco, Maselli, & Severi, 2015; Grenham, Clarke, Cryan, & Dinan, 2011). Largely recognized as a bidirectional relationship (e.g., Rhee, Pothoulakis, & Mayer, 2009), research continues to uncover the true complexities of this communication network which Rea et al. (2016) more accurately define as a multidirectional relationship. It is multidirectional in the sense that each component of this extensive communication network has the ability to moderate and manipulate the function of the other systems involved. Communication between the brain and the gut is maintained via a complex network including the CNS, autonomic nervous system (ANS), enteric nervous system (ENS), hypothalamic–pituitary–adrenal (HPA) axis, neural, endocrine and immune systems (e.g., Carabotti et al., 2015; Cryan & Dinan, 2012; Mayer, 2011; Moloney, Desbonnet, Clarke, Dinan, & Cryan, 2014). Essentially, the BGMA provides a network for signals from

the brain to influence the motor, sensory, and secretory functions of the gut while simultaneously allowing signals and metabolites from the GM to influence brain development, biochemistry, function, and behavior (e.g., Cryan & O'Mahony, 2011; Grenham et al., 2011; Marques et al., 2014). This communication system presents an exciting and novel target for psychological intervention, providing a deeper understanding of the biological underpinnings of psychological illnesses. It is hoped that developing a greater understanding of the BGMA as it relates to the Four Ps of case formulation will provide psychologists with an additional tool in the treatment of their clients.

1.2 | Is diet the chicken, or the egg?

The relationship between diet and GM is an intriguing one, ironically reminiscent of the idiom regarding which came first, the chicken or the egg. Once thought to be unidirectional (diet influencing the composition of the microbiota), recent evidence suggests the relationship could in fact be bidirectional, with microbes also being able to influence food choice and dietary-related behaviors (Alcock, Maley, & Aktipis, 2014).

Diet is arguably one of the most important environmental factors in shaping the composition and metabolic activities of GM (De Filippo et al., 2017; Garcia-Mantrana, Selma-Royo, Alcantara, & Collado, 2018; Voreades, Kozil, & Weir, 2014). As such, diet must be taken into consideration when discussing potential interventions involving GM. An extensive review of the relationship between diet and GM is beyond the scope of this paper, but can be found elsewhere (e.g., Sheflin, Melby, Carbonero, & Weir, 2017; Singh et al., 2017; Wu et al., 2011). Here, diet is discussed insofar as to highlight to psychologists the importance of this environmental factor in the formulation and treatment of their client's presenting problem. While it is not being suggested that psychologists become well versed in dietetics, gathering general information on a client's diet may provide further insight into the development and perpetuation of their presenting problem, and presents as an additional arm to a multidisciplinary and holistic treatment approach.

Both the content and diversity of an individual's diet are believed to be important in maintaining a well-balanced GM (Heiman & Greenway, 2016; Oriach, Robertson, Stanton, Cryan, & Dinan, 2016). Diet quality has also been highlighted as a potential risk or protective factor for conditions such as depression (Jacka et al., 2017; Koopman & El Aidy, 2017; Lai et al., 2014). In regards to dietary content, the industrial revolution saw a significant increase in highly processed cereals rich in carbohydrates, refined sugars, sodium, omega-6, and trans-fatty acids. Concurrently, potassium, complex carbohydrates, fiber, omega-3, and unsaturated fatty acids were considerably reduced (Rubio-Ruiz, Peredo-Escárcega, Cano-Martínez, & Guarner-Lans, 2015). These changes are reflective of what is today termed the "Western-style diet," one that has been shown to impair immune function and promote inflammation (Myles, 2014). This is concerning given that inflammation is believed to underlie and perpetuate many, if not all, psychological and neurodegenerative illnesses (Almond, 2013; Miller & Raison, 2016; Rea et al., 2016). Worryingly, dietary diversity has been further reduced over the past 50 years with an ever increasing preference for convenience and taste (Glanz, Basil, Maibach, Goldberg, & Snyder, 1998; Heiman & Greenway, 2016; Poti, Mendez, Ng, & Popkin, 2015). Essentially, this means that current human diets are not providing GM with the resources they require to perform their myriad of complex tasks involved in host homeostasis and consequently health and disease. Adding support to this contention, Jacka et al. (2017) conducted a clinical trial which demonstrated that adherence to a modified Mediterranean diet resulted in significantly greater improvement in depression ratings from baseline compared to a social support control group.

While diet is one way through which a host can modulate their GM, microbes are themselves able to influence eating behaviors of their host (Alcock et al., 2014). Microbes in the gut must cooperate and share limited resources (space and nutrients) to promote stable coexistence and ecological diversity (Allen & Nowak, 2013). This means that these microorganisms are under selective pressure to ensure their own survival and must therefore compete for available resources (Hibbing, Fuqua, Parsek, & Peterson, 2010). As such, microbes are proposed to manipulate the eating behavior of the host by either generating cravings for foods that they thrive on or those which suppress their competitors, or by influencing mood which leads to the intake of foods that enhance that species' fitness (Alcock et al., 2014; Leitao-Goncalves et al., 2017).

1.2.1 | Dietary-derived short-chain fatty acids

A continued loss of fiber from the Western diet will inevitably lead to continued depletion of short-chain fatty acids (SCFAs; Broussard & Devkota, 2016) with downstream effects on the development and perpetuation of psychological illnesses through their immunoregulatory effects (Rogers et al., 2016). SCFAs (acetic, butyric, and propionic acids in particular) are one of the main metabolites of GM (Carabotti et al., 2015; Smith et al., 2013). They are the end products of dietary fiber fermentation and have been shown to have many beneficial effects on host health (Bourassa, Alim, Bultman, & Ratan, 2016; den Besten et al., 2013).

In the brain, SCFAs demonstrate neuroprotective properties (Sun et al., 2015) with butyrate in particular having a protective effect on psychological and neurodegenerative disorders (Bourassa et al., 2016). Peripherally, SCFAs are believed to influence the size and function of regulatory T cells which play a crucial role in regulating inflammation and immune homeostasis (Hakansson & Molin, 2011; Smith et al., 2013). Additionally, SCFAs (together with enzymes also produced by the GM) enhance intestinal barrier functioning through their regulation of tight junction (TJ) proteins (e.g., Anderson et al., 2010; Bischoff et al., 2014; Peng, Li, Green, Holzman, & Lin, 2009). Abnormal intestinal permeability (leaky gut) results in increased translocation of toxins and GM across the epithelial barrier which consequently trigger an inflammatory immune response that can dysregulate ENS and systemic immune functioning (Berkes, Viswanathan, Savkovic, & Hecht, 2003; Carabotti et al., 2015; Fasano, 2012; Smith et al., 2013).

This immune response is believed to be the instigator of resultant symptom expression including psychological disorders such as depression (e.g., Maes et al., 2013; Mulak & Bonaz, 2015; Sheedy et al., 2009).

Also via their influence on TJ proteins, SCFAs are believed to regulate the permeability of the blood–brain barrier (BBB; Braniste et al., 2014). Dysregulation of the BBB has been associated with neuropsychological conditions including Alzheimer's disease (Kuhnke et al., 2007) and autism (Fiorentino et al., 2016). Schoknecht and Shalev (2012) suggest that depression and schizophrenia may also be related to BBB dysfunction. Although further research is needed, these associations are highly plausible given the BBB is responsible for regulating access of circulating macromolecules and potential neurotoxins to the brain (Fiorentino et al., 2016; Patel & Frey, 2015). As evidence of microbial involvement, Braniste et al. (2014) found that germ-free (GF) mice (those devoid of bacterial colonization and therefore lacking conventional gut flora) have increased BBB permeability compared to specific pathogen-free (SPF) mice that have conventional GM colonization free of any known pathogens. Colonization of GF mice with known SCFA-producing bacterial strains (*Clostridium tyrobutyricum* and *Bacteroides thetaiotaomicron*) was found to normalize BBB function (Braniste et al., 2014). There is also evidence to suggest that SCFAs are involved in glucose metabolism, reducing adiposity, appetite regulation, and energy homeostasis (Byrne, Chambers, Morrison, & Frost, 2015; Chambers et al., 2015; Kondo, Kishi, Fushimi, Ugajin, & Kaga, 2009; Morrison & Preston, 2016).

1.3 | Behavior

Studies using GF mice have provided the greatest depth of information regarding the influence of GM on host behavior. Such preclinical work gives useful insights into the physiological mechanisms through which the BGMA functions. For example, GF mice demonstrate altered expression of brain-derived neurotrophic factor (BDNF) and SCFA while also exhibiting altered HPA axis functioning, anxious and depressive behaviors, and social functioning (Arentsen, Raith, Qian, Forssberg, & Diaz Heijtz, 2015; Luczynski et al., 2016; Neufeld, Kang, Bienenstock, & Foster, 2011; Sudo et al., 2004). In both animal and human models, manipulation of GM has also been demonstrated to alter levels of stress hormones corticotropin-releasing factor (CRF) and cortisol (Yarandi, Peterson, Treisman, Moran, & Pasricha, 2016). Many of these abnormalities have been shown to be rectified by colonization with the feces from SPF mice or with specific probiotics (e.g., Bercik et al., 2011; Desbonnet et al., 2010; Sudo et al., 2004). However, Bravo et al. (2011) found that ingestion of probiotics was only beneficial in mice which had an intact vagus nerve, demonstrating the importance of the vagal pathway in brain–gut communication. Additionally, Sudo et al. (2004) demonstrated that recolonization was only effective if it occurred within a critical period, providing evidence for a fundamental role of GM in the development of crucial systems involved in behavioral outcomes.

A recently proposed way in which GM may manipulate host behavior is via their relationship with personality traits. Kim et al. (2018) found an increased abundance of *Gammaproteobacteria* in those with high neuroticism, as well as those with low extraversion. Low conscientiousness was associated with an increased abundance of *Proteobacteria* and a decreased abundance of *Lachnospiraceae* while those with high levels of openness demonstrated greater phylogenic diversity and richness (Kim et al., 2018). As this was the first study to have investigated the link between GM and personality directly, further research is required to elucidate these relationships. The relationship between personality and GM presents as an intriguing area of exploration, given that personality traits have a strong association with behavioral patterns in addition to physiological and psychological health outcomes (e.g., Ferguson, 2013; Kim et al., 2018; Srivastava & Das, 2015).

Additional evidence linking GM composition and behavior comes from the study of patients following gastric bypass surgery. Behavioral changes following gastric bypass surgery include patients feeling less hungry and having a preference for healthier foods (Behary & Miras, 2015) which is likely related to changes in neural responses to food (particularly high-calorie foods) in key areas of the mesolimbic reward pathway (Ochner et al., 2011, 2012). It is tempting to speculate that these changes are associated with the compositional changes in GM following gastric bypass surgery (Furet et al., 2010; Liou et al., 2013; Zhang et al., 2009). Additionally, improvements in quality of life and levels of depression have been shown to persist two years after surgery (Karlsson, Sjostrom, & Sullivan, 1998; Mokhber, Shaghayegh, Talebi, & Tavassoli, 2016). These improvements may be the result of reduced adipose tissue which has downstream effects on GM and their role in inflammation and other related functions. While causational evidence is currently unavailable, correlational research linking GM and mood (Jiang et al., 2015) suggest that changes in GM composition following gastric bypass surgery may also have a direct influence on mood. Improvements in mood may consequently encourage healthier food choices and eating behaviors (Christensen & Brooks, 2006), exemplifying the potential for a cyclical relationship involving diet, GM, and mood that is beneficial to overall health.

Given the possible role of GM in eating behaviors, and their ability to influence hunger and satiety (Cani et al., 2009) and neuropeptide and endocrine regulation (Holzer & Farzi, 2014), a relatively new line of inquiry has emerged investigating GM involvement in eating disorders which are traditionally recognized as psychological disorders (Kleiman et al., 2015; Lam, Maguire, Palacios, & Caterson, 2017). Associations between GM composition and eating disorder psychopathology were also found by Kleiman et al. (2015), further suggesting that the GM play a role in the psychology of food choice and eating behaviors. Given that diet is a key determinant of GM composition and that eating disorders are categorized by extreme dietary changes, the GM present as a logical target for inclusion in multifaceted intervention.

While this paper focuses mainly on unconscious mechanisms underlying the relationship between GM and psychological outcomes (such as interoceptive processes and neurotransmitter production), it

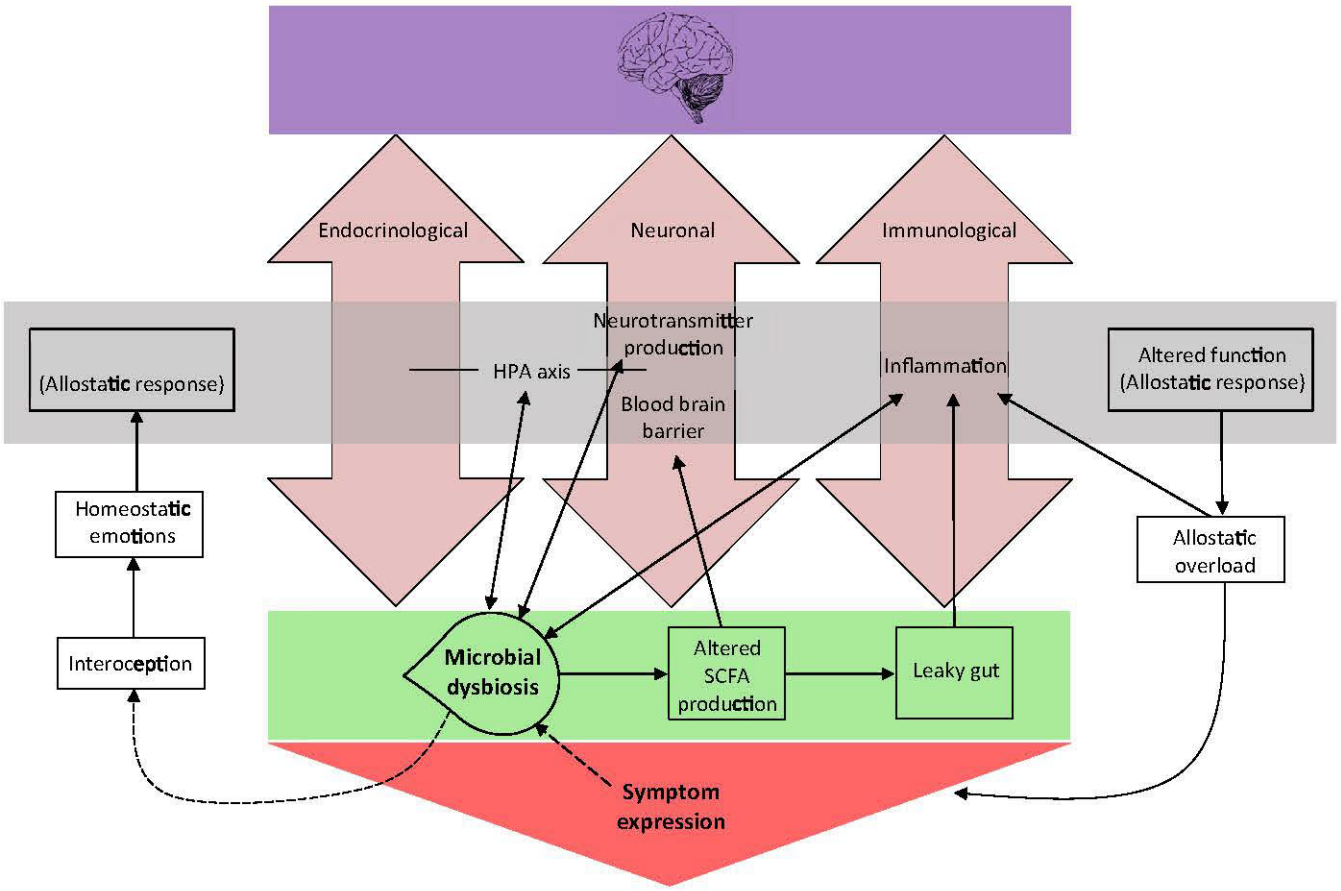

FIGURE 1 Factors influencing the multidirectional communication between the brain and the gut. Double-headed arrows demonstrate a bidirectional relationship, with broken arrows demonstrating proposed but not yet established relationships. The figure demonstrates the three main well-established pathways of communication between the brain and the gut, being endocrinological, neuronal, and immunological. The figure also illustrates the bidirectional relationships between microbial dysbiosis and the HPA axis, neurotransmitter production, the function of the blood–brain barrier, and inflammation which are believed to alter their functioning as an allostatic response to homeostatic emotions. It is proposed that microbial dysbiosis itself is able to be detected via the interoceptive system which then triggers these homeostatic emotions

is acknowledged that conscious mechanisms also have potential psychological implications. For example, gastrointestinal symptoms can be noticeably unpleasant and, particularly in IBS sufferers, can lead to impairment in daily functioning (Ballou, Bedell, & Keefer, 2015), anxiety and depression (Roohafza et al., 2016), avoidance behaviors (Van Oudenhove et al., 2016), and poor quality of life (Canavan, West, & Card, 2015). Conscious mechanisms can also lead to positive psychological outcomes as exemplified by patients following gastric bypass surgery. For example, noticeable changes in body composition can result in more positive body image which in itself is related to psychological well-being, particularly after body contouring surgery (Jumbe, Hamlet, & Meyrick, 2017; Sarwer & Steffen, 2015; Song et al., 2016). These changes can then encourage long-term weight loss maintenance behavior (Palmeira et al., 2010).

1.4 | Neurotransmitters

Perhaps the most obvious association between GM and psychological illnesses is the ability of GM to manipulate the production and action of several key neurotransmitters (e.g., Anderson & Maes, 2015; Lyte, 2011; O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). GM regulate the metabolism and concentration of amino acids which serve as precursors for several neurotransmitters including gamma-aminobutyric acid (GABA), serotonin, melatonin, and dopamine, among others (Clarke et al., 2014; Evrensel & Ceylan, 2015; Jenkins, Nguyen, Polglaze, & Bertrand, 2016; Zagajewski et al., 2012). As such, it is highly plausible that GM are able to influence brain chemistry, which consequentially regulates cognition, mood, and behavior. As depicted in Figure 1, demonstrating bidirectionality, GM can also be directly affected by neurochemicals which alter bacterial growth and pathogenicity (Lyte, 2011). Table 1 displays some of the key neurotransmitters synthesized by GM whose dysregulation is associated with psychological disorders. While neurotransmitters produced in the gut may not directly influence brain chemistry as they do not pass through the BBB, they are able to influence the CNS through mechanisms including direct stimulation of the vagus nerve, as well as using indirect circulatory and immune pathways (Sampson & Mazmanian, 2015). For example, tryptophan, the precursor molecule

TABLE 1 Gut bacteria associated with the synthesis of key neurotransmitters

Genus/species	Neurotransmitter (precursor)	CNS effect	Peripheral effect	Psychiatric conditions related to dysregulation	References
Candida; Streptococcus; Escherichia; Enterococcus; Lactobacillus bulgaricus	Serotonin (tryptophan)	Motor control, cerebellar regulation, synaptogenesis, addiction, emotion, memory, stress	Circadian rhythm, gut motility, body temperature, visceral pain, appetite, modulation of immune response	Depression, IBS, autism, Down's syndrome	Arreola et al., (2015), Gulesserian, Engidawork, Cairns, and Lubec (2000), Halford and Blundell, (2000), Hood et al. (2006), Jenkins et al. (2016), Leonard, (2010), Mazzoli and Pessione (2016), Marks et al. (2009), Meneses and Liy-Salmeron (2012), Müller and Homberg (2015), Rogers et al. (2016), Stasi, Rosselli, Zignego, Laffi, and Milani (2014), Warren and Singh, (1996), Whitaker-Azmitia (2001)
Corynebacterium glutamicum; Lactobacillus plantarum; Lactobacillus paracasei; Lactobacillus lactis; Brevibacterium lactofermentum; Brevibacterium flavum	L-glutamate	Excitatory, brain development, synaptic plasticity		Generalized anxiety disorder, depression, bipolar, schizophrenia, neurodegeneration	Abdou et al. (2006), Boonstra et al. (2015), Cherlyn et al. (2010), Femenía, Gómez-Galán, Lindskog, and Magara (2012), Hyland and Cryan (2010), Meldrum (2000), Yoto et al. (2012)
Lactobacillus; Bifidobacterium; Escherichia coli; Pseudomonas	GABA (L-glutamate)	Inhibitory, anxiolytic	Myorelaxant, moderates intestinal motility, gastric emptying, gastric acid secretion, and inhibits GI carcinogens and tumor growth		
Bacillus, Serratia, E. coli	Dopamine (L-Dopa)	Reward-motivated behavior, motor behavior, cognition, emotion	Stimulates exocrine secretion, inhibits gut motility, and modulates sodium absorption and mucosal blood flow	Schizophrenia, Parkinson's disease, depression, anxiety, addiction	Di Chiara and Bassareo (2007), Eisenhofer et al. (1997), Freestone (2013), Grace (2016), Lyte (2011), Meyer and Feldon (2009), Scheperjans et al. (2015), Shishov, Kirovskaya, Kudrin, and Oleskin (2009)
Bacillus; E. coli; Saccharomyces	Norepinephrine (dopamine)	Stress hormone, attentiveness, emotion, sleep, learning	Mediates growth and virulence of potentially pathogenic bacteria	Depression, schizophrenia	Freestone (2013), Moret and Briley (2011), Yamamoto and Hornykiewicz (2004)
(dependent on tryptophan production and serotonin synthesis)	Melatonin (serotonin)	Circadian rhythm, mood	Gastrointestinal function, protects against gut permeability, anti-inflammatory, antioxidant, analgesic	IBS, multiple sclerosis, autism, Alzheimer's, mood disorders	Fornaro, Prestia, Colicchio, and Perugi (2010), Ghorbani, Salari, Shaygannejad, and Norouzi (2013), Ortiz, Benítez-King, Rosales-Corral, Pacheco-Moisés, and Velázquez-Brizuela (2008), Veatch, Goldman, Adkins, and Malow (2015), Wong, Yang, Song, Wong, and Ho (2015)

to serotonin (which is itself the precursor to melatonin), is able to pass through the BBB and as such it is likely that metabolites of GM directly influence brain chemistry (Sampson & Mazmanian, 2015).

1.5 | Interoception and allostatic responses

It is currently unknown whether the interoceptive system is able to detect microbial dysbiosis (imbalances resulting from the under- or overabundance of certain microbial species; DeGruttola, Low, Mizoguchi, & Mizoguchi, 2016), but considering that gut microbes are an essential part of human physiology which moderate several homeostatic emotions (Craig, 2002; Mayer, Naliboff, & Craig, 2006; Noakes, 2012; Paulus & Stein, 2010) it is a strong possibility. Homeostatic emotions are background emotions that may or may not enter conscious awareness but influence an individual's energy levels, mood, and disposition (Mayer et al., 2006). Signals from internal organs, particularly the gut, continuously communicate with various regions of the brain including the limbic system, autonomic and neuroendocrine centers in the hypothalamus, brainstem, and cortex (Craig, 2002; Holzer & Farzi, 2014; Mayer & Tillisch, 2011). It is plausible that through the process of interoception, GM are able to influence human cognition, emotion, and mood through their involvement in systemic functioning through their various metabolites (Holzer, 2017; Paulus & Stein, 2010). As such, increasing rates of disease might be explained by the allostatic load hypothesis (McEwen, 1998). The allostatic load hypothesis proposes that rather than having a stable set point, body systems have a range of set points allowing them to actively adapt to environmental and internal states. Allostatic load is a term used to refer to the cumulative cost of allostasis to the body ("wear and tear"; McEwen & Wingfield, 2003). While adaptive in the short term, allostasis can become maladaptive, leading to disease, when allostatic measures are required to vary widely and frequently, or are at extreme values for long periods of time (James, 2013). Additionally, allostatic systems can become dysfunctional when they lose their ability to change or regulate change (James, 2013). The result of either of these scenarios is allostatic overload which can lead to symptom expression, as depicted in Figure 1 (Berger, Juster, & Sarnyai, 2015; McEwen, 2005; McEwen & Wingfield, 2003). The fact that research has failed to define the precise composition of a healthy GM due to immense interindividual differences (Lloyd-Price, Abu-Ali, & Huttenhower, 2016) suggests that the GM may in fact be the most allostatic system within the body. Microbial dysbiosis then could be considered an extreme and prolonged shift away from what would be considered a relatively healthy composition which loses its ability to regulate change in various other host systems and functions. It is perhaps this dysregulation that manifests itself in psychological illness.

Figure 1 depicts the three overarching pathways (endocrinological, neuronal, and immunological) of bidirectional communication between the brain and the gut, each of which is altered during a state of microbial dysbiosis. Although it remains unconfirmed, it is proposed that microbial dysbiosis can be detected via interoception which ultimately leads to altered functioning of factors (e.g., inflammation) that mediate these pathways. These alterations are believed to be an allostatic response to a deviation from a "typical" microbiome, which over time, leads to symptom expression as a result of allostatic overload. The bidirectional relationship between microbial dysbiosis and the factors which alter the three main communication pathways between the gut and the brain illustrate the multidirectional nature of the BGMA.

2 | GM THROUGH THE LENS OF A CASE FORMULATION FRAMEWORK

It is not the intention of this paper to propose that the BGMA must be at the forefront of consideration for each and every client. Instead, it is proposed that the relevance of the BGMA is determined on a case-by-case basis. The role of the BGMA in a client's presenting problem may be less relevant when there are clear social and emotional etiological factors, such as the presence of significant stressors for a client presenting with anxiety or depression. However, it is worth noting that stress can alter the composition of a person's GM (Bailey et al., 2011), which may or may not be clinically relevant, but should be considered if comorbidities are present. It may also be less relevant for clients who respond well to traditional psychological treatments such as cognitive behavioral therapy. Alternatively, cases in which the role or the BGMA may be more pertinent are when social and emotional etiological factors are less clear or absent, and also for clients who do not respond well to conventional psychological approaches. In cases where a treating clinician considers investigation of the GM to be appropriate, clients should be referred for stool sampling and analysis and an open dialogue established between the clinician and the microbiologist performing the analysis. The following information serves to highlight possible associations between GM and each of the Four Ps.

2.1 | Predisposing

As part of their first line of questioning, mental health professionals attempt to explore a client's family history of psychological illness to establish whether that individual has an underlying genetic predisposition (vulnerability) to developing a psychological condition(s). Genetic predisposition to a multitude of psychological conditions has been well established (Hyman, 2000). While research into the role of GM in genetic predisposition is scarce, several lines of evidence suggest that GM may play an integral part in a person's vulnerability to the development of psychological illnesses. Firstly, host genetics have been demonstrated to influence the composition and metabolic activities of GM (Goodrich et al., 2014; Ussar, Fujisaka, & Kahn, 2016) which have important consequences on host physiology, brain development, and health (e.g., Krishnan, Alden, & Lee, 2015; Sekirov, Russell, Antunes, & Finlay, 2010). However, the specific mechanisms behind this relationship remain unclear (Dąbrowska & Witkiewicz, 2016).

2.1.1 | Vertical transmission

Additionally, there is evidence to suggest that much like genetics are passed down from parents to offspring, GM are vertically transmitted from mother to infant (e.g., Asnicar et al., 2017; Mueller, Bakacs, Combellick, Grigoryan, & Dominguez-Bello, 2015). The transmission of microbiota from mother to infant during birth represents the most important point of microbial colonization in the infant gut, which continues over the first three years of life (Yatsunenko et al., 2012). This critical establishment period of GM is in line with the critical development period of the human host (Rea et al., 2016). It is during this time that GM play key roles in the development of the CNS, HPA axis, and immune system (Borre et al., 2014; Cox et al., 2014; Furusawa et al., 2013; Houghteling & Walker, 2015). As such, aberrations in typical colonization of GM during this critical period may also result in abnormal development of these key systems (Tamburini, Shen, Wu, & Clemente, 2016). Factors resulting in aberrations to conventional microbial colonization of the gut during this period, such as birth by cesarean section and antibiotic treatment during infancy, have been associated with increased rates of chronic and atopic diseases (Kolokotroni et al., 2012; Sevelsted, Stokholm, Bonnelykke, & Bisgaard, 2015; Vangay, Ward, Gerber, & Knights, 2015). A recent study by Polidano, Zhu, and Bornstein (2017) highlights the microbiota as a potentially important factor in the negative relationship they found between cesarean birth and a range of cognitive outcomes compared to those born vaginally.

There is a growing consensus that maternal GM may have long-term health consequences for the child (Stanislawski et al., 2017). It is therefore reasonable to suggest that vertically transmitted GM may act as a mechanism for intergenerational predisposition to psychological disorders. Further research is required as it is difficult to determine whether these intergenerational patterns are due to the vertical transmission of GM or whether they are due to learned behaviors and lifestyle factors shared among family members. This is evidenced by same-household members showing a higher similarity of GM composition to those outside of the household (Abeles et al., 2016; Song et al., 2013; Yatsunenko et al., 2012).

2.1.2 | Aging

Aging, in and of itself, can also predispose an individual to several psychological illnesses. For example, neurodegenerative illnesses, such as Alzheimer's disease, are considered an evolutionary accident occurring as a result of increased longevity (Giunta et al., 2008; Gluckman et al., 2011; Niccoli & Partridge, 2012). There is evidence to suggest that psychological disorders are also more prevalent in the elderly (e.g., Andreas et al., 2017). A contributing factor toward the increased prevalence of disease in the elderly is that the normal aging process is associated with compositional changes and reduced diversity of GM (Biagi et al., 2010). In parallel, normal aging is characterized by chronic low-grade inflammation, a phenomenon commonly referred to as "inflammaging" (Franceschi et al., 2007). It is believed that this inflammation is, at least in part, attributable to alterations in GM (Buford, 2017). Additionally, aging is associated with changes in the serotonergic system which is also believed to contribute to increased prevalence of psychological disorders in the elderly (O'Mahony et al., 2015). The serotonergic system is regulated by GM (Table 1), therefore making it vulnerable to compositional and metabolic changes (O'Mahony et al., 2015; Rogers et al., 2016).

Given the sheer complexity of the systemic functioning of the human body, there are likely to be several other processes through which GM may be involved in predisposing individuals to the development of psychological illnesses. Further research into how GM influence predisposition to psychological illness will be useful in informing preventative strategies to circumvent the growing burden of such conditions. However, GM not only play a role in predisposing an individual to certain psychological illnesses as highlighted above, but also in the onset and maintenance of those negative health outcomes.

2.2 | Precipitating and perpetuating

While psychologists tend to focus heavily on social and environmental factors involved in the onset and maintenance of psychological disorders, biological factors, such as GM composition, also contribute to these stages of disease. The maintenance of a diverse microbial ecosystem in the gut is essential for optimal host function (Moloney et al., 2014; Sekirov et al., 2010). On the other hand, reduced diversity and microbial dysbiosis have been implicated in various psychological, neurological, metabolic, functional gastrointestinal disorders, and autoimmune disease states (Blumstein, Levy, Mayer, & Harte, 2014). These include, but are not limited to, IBS (Jeffery et al., 2012; Tana et al., 2010), autism (Finegold et al., 2002), schizophrenia (Castro-Nallar et al., 2015), myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS; Fremont, Coomans, Massart, & Meirleir, 2013; Wallis, Butt, Ball, Lewis, & Bruck, 2016), multiple sclerosis (Jangi et al., 2016), dementia (Alkasir, Li, Li, Jin, & Zhu, 2017), stress (Knowles, Nelson, & Palombo, 2008), anxiety (Burch, 2016), depression (e.g., Jiang et al., 2015), obesity (Ley, Turnbaugh, Klein, & Gordon, 2006), diabetes (Larsen et al., 2010), coronary artery disease (Cui, Zhao, Hu, Zhang, & Hua, 2017; Emoto et al., 2017), and cancer (particularly colorectal cancer; Gagniere et al., 2016; Garrett, 2015). Current findings linking GM and disorders are, at this stage, associative and causative links are yet to be established. Additionally, it is unknown whether alterations in GM composition are the cause or consequence of disease. Given available evidence however, it is likely that this is a bidirectional, and cyclical, relationship as depicted in Figure 1.

2.2.1 | Stress

Stress has long been recognized as both a precipitating and perpetuating factor to various psychological conditions (Anisman & Zacharko, 1982; Corcoran et al., 2003). Given the crucial role of GM in the development and functioning of the HPA axis (Sudo, 2012), as well as their modulation of stress hormones CRF and cortisol

(Carabotti et al., 2015), the involvement of GM in the stress response is increasingly evident. This is further demonstrated by the ability to transfer stress-prone phenotypes from one mouse to another via fecal transplantation (Collins, Kassam, & Bercik, 2013). Stress also activates an inflammatory response via the promotion of inflammatory cytokines (Liu, Wang, & Jiang, 2017). Given that inflammation is recognized as underlying many psychological and neurodegenerative disorders (Almond, 2013; Miller & Raison, 2016; Rea et al., 2016), stress then appears to play a role in both the etiology and maintenance of psychological illness via biological pathways, all of which are regulated by the GM. While this is reflective of a bottom-up process whereby GM influence stress, substantial evidence also suggests the occurrence of a top-down process through which stress regulates GM composition (Bailey et al., 2011; Gur, Worly, & Bailey, 2015; Knowles et al., 2008). The fact that both top-down and bottom-up processes have been well established demonstrates the complex cyclical and multidirectional relationship between GM, stress, and psychopathology.

2.2.2 | Socioeconomic status

There is an established association between low SES and several factors negatively affecting health, one of which is poor diet (Darmon & Drewnowski, 2008; Shahar, Shai, Vardi, Shahar, & Fraser, 2005). Given that diet is the strongest environmental contributor to a properly functioning GM (e.g., De Filippo et al., 2017; Garcia-Mantrana et al., 2018), it is possible that the composition and/or metabolic activities of the GM is one of the mediating factors of the relationship between low SES and mental health outcomes. Another factor associated with low SES is lower educational achievement (Sirin, 2005) which may also be mediated by diet-related changes in GM. Individuals who eat a poor-quality diet (Western diet) have poorer performance on cognitive tasks (Khan et al., 2015) and poorer mental health (Jacka, Kremer, et al., 2011; Jacka, Mykletun, Berk, Bjelland, & Tell, 2011; Markus et al., 1998) compared to those who eat high-quality diets. In addition, an association has been found between the consumption of a Western diet and decreased left hippocampal volume (Jacka, Cherbuin, Anstey, Sachdev, & Butterworth, 2015) with hippocampal volume being related to cognition (Choi et al., 2016) and mood (Frodl et al., 2006). It is likely the GM mediate this relationship through their production of SCFAs and BDNF which have been found to be involved in neurogenesis and neuronal protection in mouse models (Canani, Di Costanzo, & Leone, 2012; Lee, Duan, & Mattson, 2002; Sun et al., 2015). Ultimately, poorer performance on cognitive tasks and poorer mental health limit a person's educational attainment (Eisenberg, Golberstein, & Hunt Justin, 2009; Fletcher, 2010; McLeod & Fettes, 2007).

2.3 | Protective

Considering the protective abilities of GM or indeed, specific microorganisms, has the potential to revolutionize the treatment of psychological conditions (Kali, 2016; Mazzoli & Pessione, 2016; Sampson & Mazmanian, 2015). The addition of GM modulation to an individual's treatment plan may be the missing link in explaining and counteracting the alarming increase in the disease burden of common mental disorders.

2.3.1 | Lifestyle factors

Healthy eating and exercise have long been promoted as being protective factors against both physiological and psychological conditions. Evidence suggests that one of the physiological mechanisms through which healthy eating and exercise affect health is the influence these factors have on the composition and metabolic activity of GM (Mika et al., 2015; Welly et al., 2016). Essentially, a high-quality diet and exercise provide the GM with the resources they require to maintain optimal host function. While the influence of diet on GM composition is widely researched, that of exercise on GM receives less attention. However, exercise has been shown to enrich microbial diversity, improve the *Bacteroidetes*-to-*Firmicutes* ratio, and support the growth of SCFA-producing bacteria which have immunomodulatory effects (Monda et al., 2017). This suggests that like diet, the beneficial outcomes of exercise may be mediated by GM which have downstream effects on mental health.

2.3.2 | Pre- and probiotics

Both pre- and probiotics have also been demonstrated to have psychotropic like effects in healthy volunteers as well as those suffering from conditions such as depression and chronic fatigue syndrome (CFS; Akkasheh et al., 2016; Messaoudi et al., 2011; Rao et al., 2009). Probiotics showing positive effects on mental health are referred to as psychobiotics (Dinan, Stanton, & Cryan, 2013). Studies have demonstrated that various probiotic formulations (mostly including *Lactobacillus* and *Bifidobacterium* species) have the ability to improve mood in healthy (no reported diagnoses of allergic, neurological, or psychological conditions) men and women (Benton, Williams, & Brown, 2007; Messaoudi et al., 2011; Steenbergen, Sellaro, Hemert, Bosch, & Colzato, 2015). In a placebo-controlled study, Yamamura et al. (2009) found a probiotic formulation to improve sleep efficacy and number of awakenings (as measured by actigraphy) in an elderly (60- to 81-year-old) sample. Moreover, a study using fMRI revealed altered activity in brain regions responsible for emotion and sensation processing in women following four weeks of probiotic formulation intake compared to women who received a placebo (Tillisch et al., 2013). Also in a placebo-controlled study, participants who took a prebiotic (Bimuno-galactooligosaccharides) daily for three weeks showed significantly lower salivary cortisol levels and decreased attentional vigilance to negative versus positive information (Schmidt et al., 2015). These findings were similar to those of a study that involved the administration of an SSRI (Murphy, Yiend, Lester, Cowen, & Harmer, 2009).

2.3.3 | Fecal microbial transplant

The increasing popularity of fecal microbial transplant (FMT) in treating various conditions including but not limited to GI disorders (Brandt & Aroniadis, 2013), Parkinson's disease (Ananthaswamy, 2011), autism (Aroniadis & Brandt, 2013), and ME/CFS (Borody, Nowak, & Finlayson, 2012) is perhaps due to the proliferation of research associating microbial dysbiosis to a range of disorders. In humans, the efficacy of FMT has been shown for conditions such as ulcerative colitis (Shi et al., 2016), but has not yet been demonstrated in treating psychological conditions. It does however offer a promising avenue given strong evidence suggesting a role of GM in the pathogenesis of psychological conditions (Evrensel & Ceylan, 2016). Animal models suggest that FMT is an effective way to ameliorate abnormal physiology and function (e.g., Sudo et al., 2004); however, clinical trials are required to demonstrate whether this approach is equally effective in humans. Additionally, further research is required into the possible risks associated with FMT. While FMT has promising therapeutic potential, Alang and Kelly (2015) present a case study of a patient who developed obesity following FMT treatment from an overweight, but otherwise healthy donor. As GM are associated with numerous physiological and psychological conditions, FMT could theoretically result in the transference of any such condition from donor to recipient (Bunnik, Aarts, & Chen, 2017). As such, it is clear that great care must be taken when screening and selecting potential donors. There is still much to learn about the associations between GM and both physiological and psychological conditions, and therefore, potential long-term risks of FMT may yet to emerge.

3 | CRITICISMS OF CONVENTIONAL TREATMENT WITH RESPECT TO GM

Conventional treatment of psychological disorders typically involves pharmacological intervention such as psychotropics and/or other medications to alter brain chemistry (e.g., Bystritsky, Khalsa, Cameron, & Schiffman, 2013; Lieberman et al., 2005). Although beneficial, such treatments may induce undesirable side effects including, but not limited to, nausea, sleep disturbance, weight gain, and sexual dysfunction (Ferguson, 2001) all of which may themselves be a result of microbe-mediated drug metabolism (Enright, Joyce, Gahan, & Griffin, 2017). Despite the ever increasing reliance on pharmacotherapy (Kallivayalil, 2008; Vozeh, 2003), disease states remain relatively stable which suggests the need for auxiliary treatment options and/or targets which take into account several body systems, including the GM. Many psychotropic drugs, known for their influence on CNS receptor function, also demonstrate antimicrobial effects (Kalayci, Demirci, & Sahin, 2014) which may have unintended consequences on the BGMA. This is particularly true of many SSRIs commonly used to treat depression and anxiety disorders.

In addition to having direct antimicrobial effects, Ayaz et al. (2015) found that sertraline (an SSRI) augments the effectiveness of several antibiotics by increasing their inhibitory zone. As such, chronic use of these drugs can induce potentially deleterious alterations in GM (Macedo et al., 2017). This may partially explain treatment resistance, although further research is needed to support this notion. Likewise, psychological interventions (e.g., cognitive behavior therapy) also target the brain via a focus on cognitions to affect behavioral change. This top-down process has demonstrated efficacy in the treatment of functional gastrointestinal disorders such as IBS (Boersma et al., 2016; Palsson & Whitehead, 2013); however, research is needed to determine whether purely psychological interventions can enact changes in GM. By continuing to treat various disorders and symptoms through pharmacological intervention without considering the etiology of initial chemical imbalances, it is unlikely that rates of morbidity will decline. The net effect of ignoring etiology at the expense of treatment is therefore an increased burden upon individuals who are affected by disease, and wider society.

4 | REDEFINING WHO WE ARE

There is currently a shift away from thinking of host–microbe interactions in such binary terms toward appreciating the complexity of the relationship between the two. Binary distinctions between host and microbiota remain useful only in so far as to aid our understanding of the role of GM in psychological well-being, which is still in its infancy. However, emerging nomenclature such as "holobiont" acknowledges that GM are not a separate entity but are instead an integral and inseparable part of human biology (Schnorr, Sankaranarayanan, Lewis, & Warinner, 2016; Theis et al., 2016). This concept is supported by the coevolution of humans and their microbes. The concept that the ratio of bacterial cells to human cells is approximately 1:1 (recently revised down from previous estimations of 10:1; Sender, Fuchs, & Milo, 2016) pays homage to the importance of respecting GM in the conceptualization of human health and well-being. This reconceptualization of what makes us human also provides a biological and tangible basis for explaining and treating psychological illnesses that can often be considered abstract.

5 | CHALLENGES AND THE WAY FORWARD

Understanding the multidirectional relationship between GM and the nervous system is hindered by its inherent complexity (Mazzoli & Pessione, 2016). However, while still in its infancy, interdisciplinary research has uncovered novel ways of conceptualizing disease. While theoretically relevant, research into the BGMA also has important practical implications, offering a more holistic approach to treatment and prevention of psychological illness. While this new field of research is promising, it remains unclear which factors, and in which combination, alter the balance between symptomatic and asymptomatic outcomes.

The sheer number of confounding variables makes it difficult to establish causational links between GM and symptomatology. However, as understanding of the GM advances, so too will research methodology and technology. It is only with continued research into the link between GM and psychological illness that we will be able to elucidate the true extent to which our resident microbes contribute to mental health. As the majority of studies regarding the role of GM in both physiological and psychological health and disease have been conducted using animal models, clinical trials with human samples are imperative to the advancement of knowledge and ultimately practical application.

As the burgeoning research into the relationship between GM and psychological health outcomes gathers momentum, so too does the call to action for psychologists to embrace the microbiome as a potential factor in explaining, treating, and preventing mental illness. This is an important paradigm shift which must occur within the discipline of psychology in order to keep up to date with the most current and complete knowledge of the human body and mind which translates into providing the best possible care for clients. While it is unnecessary and impractical to expect psychologists to develop a detailed understanding of the influence of GM on mental health, it is important that the role of the GM is acknowledged, especially in the absence of clear social and emotional factors. Facilitating this paradigm shift may require change at a "grass roots" level, where psychobiology is better integrated into higher education psychology degrees. Additionally, professional development courses regarding the role of the GM in mental health should be established and promoted to current practicing psychologists who can use this information to provide more complete care for their clients.

The fact that GM are able to influence psychological functioning is an exciting and encouraging prospect, which begs for multidisciplinary approaches to both research and practice. In terms of practical implications, increasing our understanding of the mechanisms that mediate communication processes between GM and host has the potential to inform strategies to limit the damaging aspects of this communication. This will provide new avenues of treatment for a wide range of symptoms and disorders (Freestone, 2013) as well as to promote good health. This will require a substantial shift away from the reductionist approaches that see us working exclusively in our specific field. This is not to suggest that psychologists should become expert in areas outside of their field, but instead to understand and acknowledge that the best way forward is a multidisciplinary approach to the treatment and prevention of mental illness. A shift toward a multidisciplinary, and therefore more holistic, approach will provide an opportunity to better understand the etiology of disease which requires the expertise of several disciplines and a consideration of key body systems, including the GM, as intertwined and inseparable. In light of the evidence research has provided thus far, psychologists working as part of multidisciplinary teams with other professionals such as nutritionists, gastroenterologists, and microbiologists must seriously consider the inclusion of dietary plans, pre- and probiotics, and potentially even FMT in the treatment plans of their clients, in conjunction with conventional psychological treatments.

It is not the contention of this paper to claim that ameliorating gut health is the panacea to all psychological disorders and symptomatology. Instead, it is to demonstrate the complex interconnectivity between multiple body systems in disease processes, from etiology through to treatment, and ideally prevention. In support of the call to action by Allen et al. (2017), the discipline of psychology must shift away from its CNS-centric conceptualization of disease and symptom-centered disease treatment models toward a multidisciplinary approach to treatment and prevention. This paradigm shift will empower psychologists to better treat and care for their clients. A multidisciplinary approach where healthcare professionals across a variety of disciplines have a united approach to treatment and prevention will also empower the public to better understand and take control of their physiological and psychological health. It is only through this shared awareness that the healthcare community can make inroads into improving the mental health of current and future generations.

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

Abdou, A. M., Higashiguchi, S., Horie, K., Kim, M., Hatta, H., & Yokogoshi, H. (2006). Relaxation and immunity enhancement effects of gamma-aminobutyric acid (GABA) administration in humans. *BioFactors*, *26*(3), 201–208.

Abeles, S. R., Jones, M. B., Santiago-Rodriguez, T. M., Ly, M., Klitgord, N., Yooseph, S., … Pride, D. T. (2016). Microbial diversity in individuals and their household contacts following typical antibiotic courses. *Microbiome*, *4*(1), 39. https://doi.org/10.1186/s40168-016-0187-9

Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., Taghizadeh, M., … Esmaillzadeh, A. (2016). Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. *Nutrition*, *32*(3), 315–320. https://doi.org/10.1016/j.nut.2015.09.003

Alang, N., & Kelly, C. R. (2015). Weight gain after fecal microbiota transplantation. *Open Forum Infectious Diseases*, *2*(1), ofv004. https://doi.org/10.1093/ofid/ofv004

Alcock, J., Maley, C. C., & Aktipis, C. A. (2014). Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. *BioEssays*, *36*(10), 940–949. https://doi.org/10.1002/bies.201400071

Alkasir, R., Li, J., Li, X., Jin, M., & Zhu, B. (2017). Human gut microbiota: The links with dementia development. *Protein Cell*, *8*(2), 90–102. https://doi.org/10.1007/s13238-016-0338-6

Allen, A. P., Dinan, T. G., Clarke, G., & Cryan, J. F. (2017). A psychology of the human brain-gut-microbiome axis. *Social and Personality Psychology Compass*, *11*(4), e12309. https://doi.org/10.1111/spc3.12309

Allen, B., & Nowak, M. A. (2013). Cooperation and the fate of microbial societies. *PLoS Biology*, *11*(4), e1001549. https://doi.org/10.1371/journal.pbio.1001549

Almond, M. (2013). Depression and inflammation: Examining the link. *Current Psychiatry*, *12*(6), 24–32.

Ananthaswamy, A. (2011). Faecal transplant eases symptoms of Parkinson's disease. *New Journal of Science*, *209*, 8–9.

Anderson, G., & Maes, M. (2015). The gut–brain axis: The role of melatonin in linking psychiatric, inflammatory and neurodegenerative conditions. *Advances in Integrative Medicine*, *2*(1), 31–37. https://doi.org/10.1016/j.aimed.2014.12.007

Anderson, R. C., Cookson, A. L., McNabb, W. C., Park, Z., McCann, M. J., Kelly, W. J., & Roy, N. C. (2010). Lactobacillus plantarum MB452 enhances the function of the intestinal barrier by increasing the expression levels of genes involved in tight junction formation. *BMC Microbiology*, *10*, 316. https://doi.org/10.1186/1471-2180-10-316

Andreas, S., Schulz, H., Volkert, J., Dehoust, M., Sehner, S., Suling, A., … Härter, M. (2017). Prevalence of mental disorders in elderly people: The European MentDis_ICF65+ study. *The British Journal of Psychiatry*, *210*(2), 125–131. https://doi.org/10.1192/bjp.bp.115.180463

Angst, J., Merikangas, K. R., & Preisig, M. (1997). Subthreshold syndromes of depression and anxiety in the community. *Journal of Clinical Psychiatry*, *58*(Suppl 8), 6–10.

Anisman, H., & Zacharko, R. (1982). Depression: The predisposing influence of stress. *Behavioral and Brain Sciences*, *5*(1), 89–99. https://doi.org/10.1017/S0140525X00010633

Arentsen, T., Raith, H., Qian, Y., Forssberg, H., & Diaz Heijtz, R. (2015). Host microbiota modulates development of social preference in mice. *Microbial Ecology in Health & Disease*, *26*, 29719. https://doi.org/10.3402/mehd.v26.29719

Aroniadis, O. C., & Brandt, L. J. (2013). Fecal microbiota transplantation: Past, present and future. *Current Opinion in Gastroenterology*, *29*(1), 79–84. https://doi.org/10.1097/MOG.0b013e32835a4b3e

Arreola, R., Becerril-Villanueva, E., Cruz-Fuentes, C., Velasco-Velázquez, M. A., Garcés-Alvarez, M. E., Hurtado-Alvarado, G., … Pavón, L. (2015). Immunomodulatory effects mediated by serotonin. *Journal of Immunology Research*, *2015*, 354957. https://doi.org/10.1155/2015/354957

Asnicar, F., Manara, S., Zolfo, M., Truong, D. T., Scholz, M., Armanini, F., … Segata, N. (2017). Studying vertical microbiome transmission from mothers to infants by strain-level metagenomic profiling. *mSystems*, *2*(1), e00164–16. https://doi.org/10.1128/mSystems.00164-16

Ayaz, M., Subhan, F., Ahmed, J., Khan, A.-U., Ullah, F., Ullah, I., … Hussain, S. (2015). Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. *Journal of Biological Research-Thessaloniki*, *22*(1), 4. https://doi.org/10.1186/s40709-015-0028-1

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity*, *25*(3), 397–407. https://doi.org/10.1016/j.bbi.2010.10.023

Ballou, S., Bedell, A., & Keefer, L. (2015). Psychosocial impact of irritable bowel syndrome: A brief review. *World Journal of Gastrointestinal Pathophysiology*, *6*(4), 120–123. https://doi.org/10.4291/wjgp.v6.i4.120

Behary, P., & Miras, A. D. (2015). Food preferences and underlying mechanisms after bariatric surgery. *The Proceedings of the Nutrition Society*, *74*(4), 419–425. https://doi.org/10.1017/s0029665115002074

Benton, D., Williams, C., & Brown, A. (2007). Impact of consuming a milk drink containing a probiotic on mood and cognition. *European Journal of Clinical Nutrition*, *61*(3), 355–361. https://doi.org/10.1038/sj.ejcn.1602546

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., … Verdu, E. F. (2011). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterology and Motility*, *23*(12), 1132–1139. https://doi.org/10.1111/j.1365-2982.2011.01796.x

Berger, M., Juster, R. P., & Sarnyai, Z. (2015). Mental health consequences of stress and trauma: Allostatic load markers for practice and policy with a focus on Indigenous health. *Australas Psychiatry*, *23*(6), 644–649. https://doi.org/10.1177/1039856215608281

Berkes, J., Viswanathan, V. K., Savkovic, S. D., & Hecht, G. (2003). Intestinal epithelial responses to enteric pathogens: Effects on the tight junction barrier, ion transport, and inflammation. *Gut*, *52*(3), 439–451. https://doi.org/10.1136/gut.52.3.439

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., … De Vos, W. (2010). Through ageing, and beyond: Gut microbiota and inflammatory status in seniors and centenarians. *PLoS ONE*, *5*(5), e10667. https://doi.org/10.1371/journal.pone.0010667

Bischoff, S. C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J.-D., Serino, M., … Wells, J. M. (2014). Intestinal permeability – a new target for disease prevention and therapy. *BMC Gastroenterology*, *14*(1), 189. https://doi.org/10.1186/s12876-014-0189-7

Blumstein, D. T., Levy, K., Mayer, E., & Harte, J. (2014). Gastrointestinal dysbiosis. *Evolution, Medicine, and Public Health*, *2014*(1), 163. https://doi.org/10.1093/emph/eou029

Boersma, K., Ljótsson, B., Edebol-Carlman, H., Schrooten, M., Linton, S. J., & Brummer, R. J. (2016). Exposure-based cognitive behavioral therapy for irritable bowel syndrome. A single-case experimental design across 13 subjects. *Cognitive Behaviour Therapy*, *45*(6), 415–430. https://doi.org/10.1080/16506073.2016.1194455

Boonstra, E., de Kleijn, R., Colzato, L. S., Alkemade, A., Forstmann, B. U., & Nieuwenhuis, S. (2015). Neurotransmitters as food supplements: The effects of GABA on brain and behavior. *Frontiers in Psychology*, *6*, 1520. https://doi.org/10.3389/fpsyg.2015.01520

Borody, T. J., Nowak, A., & Finlayson, S. (2012). The GI microbiome and its role in chronic fatigue syndrome: A summary of bacteriotherapy. *Journal of the Australasian College of Nutritional and Environmental Medicine*, *31*(3), 3–8.

Borre, Y. E., O'Keeffe, G. W., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2014). Microbiota and neurodevelopmental windows: Implications for brain disorders. *Trends in Molecular Medicine*, *20*(9), 509–518. https://doi.org/10.1016/j.molmed.2014.05.002

Bourassa, M. W., Alim, I., Bultman, S. J., & Ratan, R. R. (2016). Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health? *Neuroscience Letters*, *625*, 56–63. https://doi.org/10.1016/j.neulet.2016.02.009

Brandt, L. J., & Aroniadis, O. C. (2013). An overview of fecal microbiota transplantation: Techniques, indications, and outcomes. *Gastrointestinal Endoscopy*, *78*(2), 240–249. https://doi.org/10.1016/j.gie.2013.03.1329

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Toth, M., … Pettersson, S. (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Science Translational Medicine*, *6*(263), 263ra158. https://doi.org/10.1126/scitranslmed.3009759

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., … Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences USA*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Broussard, J. L., & Devkota, S. (2016). The changing microbial landscape of Western society: Diet, dwellings and discordance.

Molecular Metabolism, *5*(9), 737–742. https://doi.org/10.1016/j.molmet.2016.07.007

Buford, T. W. (2017). (Dis)Trust your gut: The gut microbiome in age-related inflammation, health, and disease. *Microbiome*, *5*(1), 80. https://doi.org/10.1186/s40168-017-0296-0

Bunnik, E. M., Aarts, N., & Chen, L. A. (2017). Physicians must discuss potential long-term risks of fecal microbiota transplantation to ensure informed consent. *The American Journal of Bioethics*, *17*(5), 61–63. https://doi.org/10.1080/15265161.2017.1299816

Burch, J. D. (2016). Intestinal infection associated with future onset of an anxiety disorder: Results of a nationally representative study. *Brain, Behaviour, and Immunity*, *57*, 222–226. https://doi.org/10.1016/j.bbi.2016.05.014

Byrne, C. S., Chambers, E. S., Morrison, D. J., & Frost, G. (2015). The role of short chain fatty acids in appetite regulation and energy homeostasis. *International Journal of Obesity*, *39*(9), 1331–1338. https://doi.org/10.1038/ijo.2015.84

Bystritsky, A., Khalsa, S. S., Cameron, M. E., & Schiffman, J. (2013). Current diagnosis and treatment of anxiety disorders. *Pharmacology & Therapeutics*, *38*(1), 30–57.

Campell, A. W. (2014). Autoimmunity and the gut. *Autoimmune Diseases*, *2014*, 1–12. https://doi.org/10.1155/2014/152428

Canani, R. B., Di Costanzo, M., & Leone, L. (2012). The epigenetic effects of butyrate: Potential therapeutic implications for clinical practice. *Clinical Epigenetics*, *4*(1), 4. https://doi.org/10.1186/1868-7083-4-4

Canavan, C., West, J., & Card, T. (2015). Change in quality of life for patients with irritable bowel syndrome following referral to a gastroenterologist: A cohort study. *PLoS ONE*, *10*(10), e0139389. https://doi.org/10.1371/journal.pone.0139389

Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., … Delzenne, N. M. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *American Journal of Clinical Nutrition*, *90*(5), 1236–1243. https://doi.org/10.3945/ajcn.2009.28095

Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Annals of Gastroenterology*, *28*(2), 203–209.

Castro-Nallar, E., Bendall, M. L., Pérez-Losada, M., Sabuncyan, S., Severance, E. G., Dickerson, F. B., … Crandall, K. A. (2015). Composition, taxonomy and functional diversity of the oropharynx microbiome in individuals with schizophrenia and controls. *PeerJ*, *3*, e1140. https://doi.org/10.7717/peerj.1140

Chambers, E. S., Viardot, A., Psichas, A., Morrison, D. J., Murphy, K. G., Zac-Varghese, S. E. K., … Frost, G. (2015). Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut*, *64*(11), 1744–1754. https://doi.org/10.1136/gutjnl-2014-307913

Cherlyn, S. Y., Woon, P. S., Liu, J. J., Ong, W. Y., Tsai, G. C., & Sim, K. (2010). Genetic association studies of glutamate, GABA and related genes in schizophrenia and bipolar disorder: A decade of advance. *Neuroscience and Biobehavioral Reviews*, *34*(6), 958–977. https://doi.org/10.1016/j.neubiorev.2010.01.002

Choi, M.-H., Kim, H.-S., Gim, S.-Y., Kim, W.-R., Mun, K.-R., Tack, G.-R., … Chung, S.-C. (2016). Differences in cognitive ability and hippocampal volume between Alzheimer's disease, amnestic mild cognitive impairment, and healthy control groups, and their correlation. *Neuroscience Letters*, *620*, 115–120. https://doi.org/10.1016/j.neulet.2016.03.044

Christensen, L., & Brooks, A. (2006). Changing food preference as a function of mood. *Journal of Psychology*, *140*(4), 293–306. https://doi.org/10.3200/jrlp.140.4.293-306

Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. F., & Dinan, T. G. (2014). Minireview: Gut microbiota: The neglected endocrine organ. *Molecular Endocrinology*, *28*(8), 1221–1238. https://doi.org/10.1210/me.2014-1108

Collins, S. M., Kassam, Z., & Bercik, P. (2013). The adoptive transfer of behavioral phenotype via the intestinal microbiota: Experimental evidence and clinical implications. *Current Opinion in Microbiology*, *16*(3), 240–245. https://doi.org/10.1016/j.mib.2013.06.004

Corcoran, C., Walker, E., Huot, R., Mittal, V., Tessner, K., Kestler, L., & Malaspina, D. (2003). The stress cascade and schizophrenia: Etiology and onset. *Schizophrenia Bulletin*, *29*(4), 671–692. https://doi.org/10.1093/oxfordjournals.schbul.a007038

Cox, L. M., Yamanishi, S., Sohn, J., Alekseyenko, A. V., Leung, J. M., Cho, I., … Blaser, M. J. (2014). Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell*, *158*(4), 705–721. https://doi.org/10.1016/j.cell.2014.05.052

Craig, A. D. (2002). How do you feel? Interoception: The sense of the physiological condition of the body. *Nature Reviews Neuroscience*, *3*(8), 655–666. https://doi.org/10.1038/nrn894

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*(10), 701–712. https://doi.org/10.1038/nrn3346

Cryan, J. F., & O'Mahony, S. M. (2011). The microbiome-gut-brain axis: From bowel to behavior. *Neurogastroenterology and Motility*, *23*(3), 187–192. https://doi.org/10.1111/j.1365-2982.2010.01664.x

Cui, L., Zhao, T., Hu, H., Zhang, W., & Hua, X. (2017). Association study of gut flora in coronary heart disease through high-throughput sequencing. *BioMed Research International*, *2017*, 3796359. https://doi.org/10.1155/2017/3796359

Dąbrowska, K., & Witkiewicz, W. (2016). Correlations of host genetics and gut microbiome composition. *Frontiers in Microbiology*, *7*, 1357. https://doi.org/10.3389/fmicb.2016.01357

Darmon, N., & Drewnowski, A. (2008). Does social class predict diet quality? *American Journal of Clinical Nutrition*, *87*(5), 1107–1117. https://doi.org/10.1093/ajcn/87.5.1107

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., … Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563. https://doi.org/10.1038/nature12820

De Filippo, C., Di Paola, M., Ramazzotti, M., Albanese, D., Pieraccini, G., Banci, E., … Lionetti, P. (2017). Diet, environments, and gut microbiota: A preliminary investigation in children living in rural and urban Burkina Faso and Italy. *Frontiers in Microbiology*, *8*, 1979. https://doi.org/10.3389/fmicb.2017.01979

DeGruttola, A. K., Low, D., Mizoguchi, A., & Mizoguchi, E. (2016). Current understanding of dysbiosis in disease in human and animal models. *Inflammatory Bowel Diseases*, *22*(5), 1137–1150. https://doi.org/10.1097/MIB.0000000000000750

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340. https://doi.org/10.1194/jlr.R036012

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179–1188. https://doi.org/10.1016/j.neuroscience.2010.08.005

Di Chiara, G., & Bassareo, V. (2007). Reward system and addiction: What dopamine does and doesn't do. *Current Opinion in Pharmacology*, *7*(1), 69–76. https://doi.org/10.1016/j.coph.2006.11.003

Dinan, T. G., Stanton, C., & Cryan, J. F. (2013). Psychobiotics: A novel class of psychotropic. *Biological Psychiatry*, *74*(10), 720–726. https://doi.org/10.1016/j.biopsych.2013.05.001

Eisenberg, D., Golberstein, E., & Hunt Justin, B. (2009). Mental health and academic success in college. *The B.E. Journal of Economic Analysis & Policy*, *9*(1), 1–37. https://doi.org/10.2202/1935-1682.2191

Eisenhofer, G., Åneman, A., Friberg, P., Hooper, D., Fåndriks, L., Lonroth, H., … Mezey, E. (1997). Substantial production of dopamine in the human gastrointestinal tract. *The Journal of Clinical Endocrinology*

& *Metabolism*, *82*(11), 3864–3871. https://doi.org/10.1210/jcem.82.11.4339

Emoto, T., Yamashita, T., Kobayashi, T., Sasaki, N., Hirota, Y., Hayashi, T., … Hirata, K.-I. (2017). Characterization of gut microbiota profiles in coronary artery disease patients using data mining analysis of terminal restriction fragment length polymorphism: Gut microbiota could be a diagnostic marker of coronary artery disease. *Heart and Vessels*, *32*(1), 39–46. https://doi.org/10.1007/s00380-016-0841-y

Enright, E. F., Joyce, S. A., Gahan, C. G., & Griffin, B. T. (2017). Impact of gut microbiota-mediated bile acid metabolism on the solubilization capacity of bile salt micelles and drug solubility. *Molecular Pharmaceutics*, *14*(4), 1251–1263. https://doi.org/10.1021/acs.molpharmaceut.6b01155

Evrensel, A., & Ceylan, M. E. (2015). The gut-brain axis: The missing link in depression. *Clinical Psychopharmacology and Neuroscience*, *13*(3), 239–244. https://doi.org/10.9758/cpn.2015.13.3.239

Evrensel, A., & Ceylan, M. E. (2016). Fecal microbiota transplantation and its usage in neuropsychiatric disorders. *Clinical Psychopharmacology and Neuroscience*, *14*(3), 231–237. https://doi.org/10.9758/cpn.2016.14.3.231

Fasano, A. (2012). Intestinal permeability and its regulation by zonulin: Diagnostic and therapeutic implications. *Clinical Gastroenterology and Hepatology*, *10*(10), 1096–1100. https://doi.org/10.1016/j.cgh.2012.08.012

Femenía, T., Gómez-Galán, M., Lindskog, M., & Magara, S. (2012). Dysfunctional hippocampal activity affects emotion and cognition in mood disorders. *Brain Research*, *1476*, 58–70. https://doi.org/10.1016/j.brainres.2012.03.053

Ferguson, E. (2013). Personality is of central concern to understand health: Towards a theoretical model for health psychology. *Health Psychology Review*, *7*(Suppl 1), S32–S70. https://doi.org/10.1080/17437199.2010.547985

Ferguson, J. M. (2001). SSRI antidepressant medications: adverse effects and tolerability. *Primary Care Companion to the Journal of Clinical Psychiatry*, *3*(1), 22–27. https://doi.org/10.4088/PCC.v03n0105

Finegold, S. M., Molitoris, D., Song, Y., Liu, C., Vaisanen, M.-L., Bolte, E., … Kaul, A. (2002). Gastrointestinal microflora studies in late-onset autism. *Clinical Infectious Diseases*, *35*(Suppl 1), S6–s16. https://doi.org/10.1086/341914

Fiorentino, M., Sapone, A., Senger, S., Camhi, S. S., Kadzielski, S. M., Buie, T. M., … Fasano, A. (2016). Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Molecular Autism*, *7*, 49. https://doi.org/10.1186/s13229-016-0110-z

Fletcher, J. M. (2010). Adolescent depression and educational attainment: Results using sibling fixed effects. *Health Economics*, *19*(7), 855–871. https://doi.org/10.1002/hec.1526

Fornaro, M., Prestia, D., Colicchio, S., & Perugi, G. (2010). A systematic, updated review on the antidepressant agomelatine focusing on its melatonergic modulation. *Current Neuropharmacology*, *8*(3), 287–304. https://doi.org/10.2174/157015910792246227

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., … Salvioli, S. (2007). Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of Ageing and Development*, *128*(1), 92–105. https://doi.org/10.1016/j.mad.2006.11.016

Freestone, P. (2013). Communication between bacteria and their hosts. *Scientifica*, *2013*, 1–15. https://doi.org/10.1155/2013/361073

Fremont, M., Coomans, D., Massart, S., & De Meirleir, K. (2013). High-throughput 16S rRNA gene sequencing reveals alterations of intestinal microbiota in myalgic encephalomyelitis/chronic fatigue syndrome patients. *Anaerobe*, *22*, 50–56. https://doi.org/10.1016/j.anaerobe.2013.06.002

Friedrich, M. (2017). Depression is the leading cause of disability around the world. *JAMA*, *317*(15), 1517. https://doi.org/10.1001/jama.2017.3826

Frodl, T., Schaub, A., Banac, S., Charypar, M., Jäger, M., Kümmler, P., … Meisenzahl, E. M. (2006). Reduced hippocampal volume correlates with executive dysfunctioning in major depression. *Journal of Psychiatry and Neuroscience*, *31*(5), 316–325.

Furet, J.-P., Kong, L.-C., Tap, J., Poitou, C., Basdevant, A., Bouillot, J.-L., … Clement, K. (2010). Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: Links with metabolic and low-grade inflammation markers. *Diabetes*, *59*(12), 3049–3057. https://doi.org/10.2337/db10-0253

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., … Ohno, H. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*, *504*(7480), 446–450. https://doi.org/10.1038/nature12721

Gagniere, J., Raisch, J., Veziant, J., Barnich, N., Bonnet, R., Buc, E., … Bonnet, M. (2016). Gut microbiota imbalance and colorectal cancer. *World Journal of Gastroenterology*, *22*(2), 501–518. https://doi.org/10.3748/wjg.v22.i2.501

Garakani, A., Win, T., Virk, S., Gupta, S., Kaplan, D., & Masand, P. S. (2003). Comorbidity of irritable bowel syndrome in psychiatric patients: A review. *American Journal of Therapeutics*, *10*(1), 61–67. https://doi.org/10.1097/00045391-200301000-00014

Garcia-Mantrana, I., Selma-Royo, M., Alcantara, C., & Collado, M. C. (2018). Shifts on gut microbiota associated to mediterranean diet adherence and specific dietary intakes on general adult population. *Frontiers in Microbiology*, *9*, 890. https://doi.org/10.3389/fmicb.2018.00890

Garrett, W. S. (2015). Cancer and the microbiota. *Science*, *348*(6230), 80–86. https://doi.org/10.1126/science.aaa4972

Ghorbani, A., Salari, M., Shaygannejad, V., & Norouzi, R. (2013). The role of melatonin in the pathogenesis of multiple sclerosis: A case-control study. *International Journal of Preventive Medicine*, *4*(Suppl 2), S180–S184.

Giunta, B., Fernandez, F., Nikolic, W. V., Obregon, D., Rrapo, E., Town, T., & Tan, J. (2008). Inflammaging as a prodrome to Alzheimer's disease. *Journal of Neuroinflammation*, *5*, 51. https://doi.org/10.1186/1742-2094-5-51

Glanz, K., Basil, M., Maibach, E., Goldberg, J., & Snyder, D. (1998). Why Americans eat what they do: Taste, nutrition, cost, convenience, and weight control concerns as influences on food consumption. *Journal of the American Dietetic Association*, *98*(10), 1118–1126. https://doi.org/10.1016/s0002-8223(98)00260-0

Gluckman, P. D., Low, F. M., Buklijas, T., Hanson, M. A., & Beedle, A. S. (2011). How evolutionary principles improve the understanding of human health and disease. *Evolutionary Applications*, *4*(2), 249–263. https://doi.org/10.1111/j.1752-4571.2010.00164.x

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., … Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, *159*(4), 789–799. https://doi.org/10.1016/j.cell.2014.09.053

Grace, A. A. (2016). Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. *Nature Reviews Neuroscience*, *17*(8), 524–532. https://doi.org/10.1038/nrn.2016.57

Grenham, S., Clarke, G., Cryan, J. F., & Dinan, T. G. (2011). Brain-gut-microbe communication in health and disease. *Frontiers in Physiology*, *2*, 94. https://doi.org/10.3389/fphys.2011.00094

Gulesserian, T., Engidawork, E., Cairns, N., & Lubec, G. (2000). Increased protein levels of serotonin transporter in frontal cortex of patients with Down syndrome. *Neuroscience Letters*, *296*(1), 53–57. https://doi.org/10.1016/S0304-3940(00)01624-4

Gur, T. L., Worly, B. L., & Bailey, M. T. (2015). Stress and the commensal microbiota: Importance in parturition and infant neurodevelopment. *Frontiers in Psychiatry*, *6*, 5. https://doi.org/10.3389/fpsyt.2015.00005

Hakansson, A., & Molin, G. (2011). Gut microbiota and inflammation. *Nutrients*, *3*(6), 637–682. https://doi.org/10.3390/nu3060637

Halford, J. C., & Blundell, J. E. (2000). Separate systems for serotonin and leptin in appetite control. *Annals of Medicine*, *32*(3), 222–232. https://doi.org/10.3109/07853890008998829

Haller, H., Cramer, H., Lauche, R., Gass, F., & Dobos, G. J. (2014). The prevalence and burden of subthreshold generalized anxiety disorder: A systematic review. *BMC Psychiatry*, *14*(128), 1–13. https://doi.org/10.1186/1471-244X-14-128

Harvey, S. B., Deady, M., Wang, M., Mykletun, A., Butterworth, P., Christensen, H., & Mitchell, P. B. (2017). Is the prevalence of mental illness increasing in Australia? Evidence from national health surveys and administrative data, 2001–2004. *Medical Journal of Australia*, *206*(11), 490–493. https://doi.org/10.5694/mja16.00295

Heiman, M. L., & Greenway, F. L. (2016). A healthy gastrointestinal microbiome is dependent on dietary diversity. *Molecular Metabolism*, *5*(5), 317–320. https://doi.org/10.1016/j.molmet.2016.02.005

Hibbing, M. E., Fuqua, C., Parsek, M. R., & Peterson, S. B. (2010). Bacterial competition: Surviving and thriving in the microbial jungle. *Nature Reviews Microbiology*, *8*(1), 15–25. https://doi.org/10.1038/nrmicro2259

Holzer, P. (2017). Interoception and gut feelings: Unconscious body signals' impact on brain function, behavior and belief processes. In H.-F. Angel, L. Oviedo, R. F. Paloutzian, A. L. C. Runehov, & R. J. Seitz (Eds.), *New approaches to the scientific study of religion: Vol. 1. Processes of believing: The acquisition, maintenance, and change in creditions* (pp. 435–442). Cham, Switzerland: Springer International Publishing.

Holzer, P., & Farzi, A. (2014). Neuropeptides and the microbiota-gut-brain axis. *Advances in Experimental Medicine and Biology*, *817*, 195–219. https://doi.org/10.1007/978-1-4939-0897-4_9

Hood, S. D., Hince, D. A., Robinson, H., Cirillo, M., Christmas, D., & Kaye, J. M. (2006). Serotonin regulation of the human stress response. *Psychoneuroendocrinology*, *31*(9), 1087–1097. https://doi.org/10.1016/j.psyneuen.2006.07.001

Houghteling, P. D., & Walker, W. A. (2015). Why is initial bacterial colonization of the intestine important to the infant's and child's health? *Journal of Pediatric Gastroenterology and Nutrition*, *60*(3), 294–307. https://doi.org/10.1097/MPG.0000000000000597

Hyland, N. P., & Cryan, J. F. (2010). A gut feeling about GABA: Focus on GABA(B) receptors. *Frontiers in Pharmacology*, *1*, 124. https://doi.org/10.3389/fphar.2010.00124

Hyman, S. E. (2000). The genetics of mental illness: Implications for practice. *Bulletin of the World Health Organization*, *78*(4), 455–463.

Jacka, F. N., Cherbuin, N., Anstey, K. J., Sachdev, P., & Butterworth, P. (2015). Western diet is associated with a smaller hippocampus: A longitudinal investigation. *BMC Medicine*, *13*, 215. https://doi.org/10.1186/s12916-015-0461-x

Jacka, F. N., Kremer, P. J., Berk, M., de Silva-Sanigorski, A. M., Moodie, M., Leslie, E. R., … Swinburn, B. A. (2011). A prospective study of diet quality and mental health in adolescents. *PLoS ONE*, *6*(9), e24805. https://doi.org/10.1371/journal.pone.0024805

Jacka, F. N., Mykletun, A., Berk, M., Bjelland, I., & Tell, G. S. (2011). The association between habitual diet quality and the common mental disorders in community-dwelling adults: The Hordaland Health study. *Psychosomatic Medicine*, *73*(6), 483–490. https://doi.org/10.1097/PSY.0b013e318222831a

Jacka, F. N., O'Neil, A., Opie, R., Itsiopoulos, C., Cotton, S., Mohebbi, M., … Berk, M. (2017). A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES' trial). *BMC Medicine*, *15*(1), 23. https://doi.org/10.1186/s12916-017-0791-y

James, G. D. (2013). Ambulatory blood pressure variation: Allostasis and adaptation. *Autonomic Neuroscience*, *177*(2), 87–94. https://doi.org/10.1016/j.autneu.2013.03.012

Jangi, S., Gandhi, R., Cox, L. M., Li, N., von Glehn, F., Yan, R., … Weiner, H. L. (2016). Alterations of the human gut microbiome in multiple sclerosis. *Nature Communications*, *7*, 12015. https://doi.org/10.1038/ncomms12015

Jeffery, I. B., O'Toole, P. W., Ohman, L., Claesson, M. J., Deane, J., Quigley, E. M., & Simren, M. (2012). An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut*, *61*(7), 997–1006. https://doi.org/10.1136/gutjnl-2011-301501

Jenkins, T. A., Nguyen, J. C., Polglaze, K. E., & Bertrand, P. P. (2016). Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients*, *8*(1), 56. https://doi.org/10.3390/nu8010056

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., … Ruan, B. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, Behavior, and Immunity*, *48*, 186–194. https://doi.org/10.1016/j.bbi.2015.03.016

Johansen, T., & Dittrich, W. H. (2013). Cognitive performance in a subclinical obsessive-compulsive sample 1: Cognitive functions. *Psychiatry Journal*, *1*, 1–10. https://doi.org/10.1155/2013/565191

Jumbe, S., Hamlet, C., & Meyrick, J. (2017). Psychological aspects of bariatric surgery as a treatment for obesity. *Current Obesity Reports*, *6*(1), 71–78. https://doi.org/10.1007/s13679-017-0242-2

Kalayci, S., Demirci, S., & Sahin, F. (2014). Determination of antimicrobial properties of picaridin and DEET against a broad range of microorganisms. *World Journal of Microbiology & Biotechnology*, *30*(2), 407–411. https://doi.org/10.1007/s11274-013-1456-4

Kali, A. (2016). Psychobiotics: An emerging probiotic in psychiatric practice. *Biomedical Journal*, *39*(3), 223–224. https://doi.org/10.1016/j.bj.2015.11.004

Kallivayalil, R. A. (2008). Are we over-dependent on pharmacotherapy? *Indian J Psychiatry*, *50*(1), 7–9. https://doi.org/10.4103/0019-5545.39750

Karlsson, J., Sjostrom, L., & Sullivan, M. (1998). Swedish obese subjects (SOS)–an intervention study of obesity. Two-year follow-up of health-related quality of life (HRQL) and eating behavior after gastric surgery for severe obesity. *International Journal of Obesity and Related Metabolic Disorders*, *22*(2), 113–126.

Kelly, J. R., Clarke, G., Cryan, J. F., & Dinan, T. G. (2016). Brain-gut-microbiota axis: Challenges for translation in psychiatry. *Annals of Epidemiology*, *26*(5), 366–372. https://doi.org/10.1016/j.annepidem.2016.02.008

Khan, N. A., Raine, L. B., Drollette, E. S., Scudder, M. R., Kramer, A. F., & Hillman, C. H. (2015). Dietary fiber is positively associated with cognitive control among prepubertal children. *Journal of Nutrition*, *145*(1), 143–149. https://doi.org/10.3945/jn.114.198457

Kim, H., Yun, Y., Ryu, S., Chang, Y., Kwon, M., Cho, J., … Kim, H. (2018). Correlation between gut microbiota and personality in adults: A cross-sectional study. *Brain, Behavior, and Immunity*, *69*, 374–385. https://doi.org/10.1016/j.bbi.2017.12.012

Kleiman, S. C., Watson, H. J., Bulik-Sullivan, E. C., Huh, E. Y., Tarantino, L. M., Bulik, C. M., & Carroll, I. M. (2015). The intestinal microbiota in acute anorexia nervosa and during renourishment: relationship to depression, anxiety, and eating disorder psychopathology. *Psychosomatic Medicine*, *77*(9), 969–981. https://doi.org/10.1097/PSY.0000000000000247

Knowles, S. R., Nelson, E. A., & Palombo, E. A. (2008). Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: A possible mechanism underlying susceptibility to illness. *Biological Psychology*, *77*(2), 132–137. https://doi.org/10.1016/j.biopsycho.2007.09.010

Kolokotroni, O., Middleton, N., Gavatha, M., Lamnisos, D., Priftis, K. N., & Yiallouros, P. K. (2012). Asthma and atopy in children born by caesarean section: Effect modification by family history of allergies – a population based cross-sectional study. *BMC Pediatrics*, *12*, 179–179. https://doi.org/10.1186/1471-2431-12-179

Kondo, T., Kishi, M., Fushimi, T., Ugajin, S., & Kaga, T. (2009). Vinegar intake reduces body weight, body fat mass, and serum triglyceride levels in obese Japanese subjects. *Bioscience, Biotechnology, and Biochemistry*, *73*(8), 1837–1843. https://doi.org/10.1271/bbb.90231

Koopman, M., & El Aidy, S. (2017). Depressed gut? The microbiota-diet-inflammation trialogue in depression. *Current Opinion in Psychiatry*, *30*(5), 369–377. https://doi.org/10.1097/yco.0000000000000350

Krishnan, S., Alden, N., & Lee, K. (2015). Pathways and functions of gut microbiota metabolism impacting host physiology. *Current Opinion in Biotechnology*, *36*, 137–145. https://doi.org/10.1016/j.copbio.2015.08.015

Kuhnke, D., Jedlitschky, G., Grube, M., Krohn, M., Jucker, M., Mosyagin, I., … Vogelgesang, S. (2007). MDR1-P-glycoprotein (ABCB1) mediates transport of Alzheimer's Amyloid-β peptides—implications for the mechanisms of Aβ clearance at the blood-brain barrier. *Brain Pathology*, *17*(4), 347–353. https://doi.org/10.1111/j.1750-3639.2007.00075.x

Lai, J. S., Hiles, S., Bisquera, A., Hure, A. J., McEvoy, M., & Attia, J. (2014). A systematic review and meta-analysis of dietary patterns and depression in community-dwelling adults. *American Journal of Clinical Nutrition*, *99*(1), 181–197. https://doi.org/10.3945/ajcn.113.069880

Lam, Y. Y., Maguire, S., Palacios, T., & Caterson, I. D. (2017). Are the gut bacteria telling us to eat or not to eat? Reviewing the role of gut microbiota in the etiology, disease progression and treatment of eating disorders. *Nutrients*, *9*(6), 602. https://doi.org/10.3390/nu9060602

Larsen, N., Vogensen, F. K., van den Berg, F. W. J., Nielsen, D. S., Andreasen, A. S., Pedersen, B. K., … Jakobsen, M. (2010). Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE*, *5*(2), e9085. https://doi.org/10.1371/journal.pone.0009085

Lee, J., Duan, W., & Mattson, M. P. (2002). Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *Journal of Neurochemistry*, *82*(6), 1367–1375. https://doi.org/10.1046/j.1471-4159.2002.01085.x

Lee, Y.-T., Hu, L.-Y., Shen, C.-C., Huang, M.-W., Tsai, S.-J., Yang, A. C., … Hung, J.-H. (2015). Risk of psychiatric disorders following irritable bowel syndrome: A nationwide population-based cohort study. *PLoS ONE*, *10*(7), e0133283. https://doi.org/10.1371/journal.pone.0133283

Leitão-Gonçalves, R., Carvalho-Santos, Z., Francisco, A. P., Fioreze, G. T., Anjos, M., Baltazar, C., … Ribeiro, C. (2017). Commensal bacteria and essential amino acids control food choice behavior and reproduction. *PLoS Biology*, *15*(4), e2000862. https://doi.org/10.1371/journal.pbio.2000862

Leonard, B. E. (2010). The concept of depression as a dysfunction of the immune system. *Current Immunology Reviews*, *6*(3), 205–212. https://doi.org/10.2174/157339510791823835

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023. https://doi.org/10.1038/4441022a

Lieberman, J. A., Stroup, T. S., McEvoy, J. P., Swartz, M. S., Rosenheck, R. A., Perkins, D. O., … Hsiao, J. K. (2005). Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *New England Journal of Medicine*, *353*(12), 1209–1223. https://doi.org/10.1056/NEJMoa051688

Linneberg, A., Nielsen, N. H., Madsen, F., Frølund, L., Dirksen, A., & Jørgensen, T. (2000). Increasing prevalence of specific IgE to aeroallergens in an adult population: Two cross-sectional surveys 8 years apart: The Copenhagen Allergy Study. *The Journal of Allergy and Clinical Immunology*, *106*(2), 247–252. https://doi.org/10.1067/mai.2000.108312

Liou, A. P., Paziuk, M., Luevano, J.-M., Machineni, S., Turnbaugh, P. J., & Kaplan, L. M. (2013). Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Science Translational Medicine*, *5*(178), 178ra141. https://doi.org/10.1126/scitranslmed.3005687

Liu, Y. Z., Wang, Y. X., & Jiang, C. L. (2017). Inflammation: The common pathway of stress-related diseases. *Frontiers in Human Neuroscience*, *11*, 316. https://doi.org/10.3389/fnhum.2017.00316

Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine*, *8*(1), 51. https://doi.org/10.1186/s13073-016-0307-y

Luczynski, P., McVey Neufeld, K. A., Oriach, C. S., Clarke, G., Dinan, T. G., & Cryan, J. F. (2016). Growing up in a bubble: Using germ-free animals to assess the influence of the gut microbiota on brain and behavior. *International Journal of Neuropsychopharmacology*, *19*(8), pyw020. https://doi.org/10.1093/ijnp/pyw020

Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. *BioEssays*, *33*(8), 574–581. https://doi.org/10.1002/bies.201100024

Macedo, D., Filho, A. J., Soares de Sousa, C. N., Quevedo, J., Barichello, T., Junior, H. V., & Freitas de Lucena, D. (2017). Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness. *Journal of Affective Disorders*, *208*, 22–32. https://doi.org/10.1016/j.jad.2016.09.012

Maes, M., Kubera, M., Leunis, J. C., Berk, M., Geffard, M., & Bosmans, E. (2013). In depression, bacterial translocation may drive inflammatory responses, oxidative and nitrosative stress (O&NS), and autoimmune responses directed against O&NS-damaged neoepitopes. *Acta Psychiatrica Scandinavica*, *127*(5), 344–354. https://doi.org/10.1111/j.1600-0447.2012.01908.x

Marks, D. M., Shah, M. J., Patkar, A. A., Masand, P. S., Park, G. Y., & Pae, C. U. (2009). Serotonin-norepinephrine reuptake inhibitors for pain control: Premise and promise. *Current Neuropharmacology*, *7*(4), 331–336. https://doi.org/10.2174/157015909790031201

Markus, C. R., Panhuysen, G., Tuiten, A., Koppeschaar, H., Fekkes, D., & Peters, M. L. (1998). Does carbohydrate-rich, protein-poor food prevent a deterioration of mood and cognitive performance of stress-prone subjects when subjected to a stressful task? *Appetite*, *31*(1), 49–65. https://doi.org/10.1006/appe.1997.0155

Marques, T. M., Cryan, J. F., Shanahan, F., Fitzgerald, G. F., Ross, R. P., Dinan, T. G., & Stanton, C. (2014). Gut microbiota modulation and implications for host health: Dietary strategies to influence the gut-brain axis. *Innovative Food Science & Emerging Technologies*, *22*, 239–247. https://doi.org/10.1016/j.ifset.2013.10.016

Marvasti, F. F., & Stafford, R. S. (2012). From sick care to health care — reengineering prevention into the U.S. system. *New England Journal of Medicine*, *367*(10), 889–891. https://doi.org/10.1056/NEJMp1206230

Mathieson, F., Collings, S., & Dowell, A. (2009). Sub-threshold mental health syndromes: Finding an alternative to the medication of unhappiness. *Journal of Primary Health Care*, *1*(1), 74–77.

Mayer, E. A. (2011). Gut feelings: The emerging biology of gut-brain communication. *Nature Reviews Neuroscience*, *12*(8), 453–466. https://doi.org/10.1038/nrn3071

Mayer, E. A., Naliboff, B. D., & Craig, A. D. (2006). Neuroimaging of the brain-gut axis: From basic understanding to treatment of functional GI disorders. *Gastroenterology*, *131*(6), 1925–1942. https://doi.org/10.1053/j.gastro.2006.10.026

Mayer, E. A., & Tillisch, K. (2011). The brain-gut axis in abdominal pain syndromes. *Annual Review of Medicine*, *62*, 381–396. https://doi.org/10.1146/annurev-med-012309-103958

Mazzoli, R., & Pessione, E. (2016). The neuro-endocrinological role of microbial glutamate and GABA signaling. *Frontiers in Microbiology*, *7*, 1934. https://doi.org/10.3389/fmicb.2016.01934

McEwen, B. S. (1998). Stress, adaptation, and disease. Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*, 33–44. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x

McEwen, B. S. (2005). Stressed or stressed out: What is the difference? *Journal of Psychiatry and Neuroscience*, *30*(5), 315–318.

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*(1), 2–15. https://doi.org/10.1016/S0018-506X(02)00024-7

McLeod, J. D., & Fettes, D. L. (2007). Trajectories of failure: The educational careers of children with mental health problems. *American Journal of Sociology*, *113*(3), 653–701. https://doi.org/10.1086/521849

Meldrum, B. S. (2000). Glutamate as a neurotransmitter in the brain: Review of physiology and pathology. *Journal of Nutrition*, *130*(4S Suppl), 1007s–1015s. https://doi.org/10.1093/jn/130.4.1007S

Mendlowicz, M. V., & Stein, M. B. (2000). Quality of life in individuals with anxiety disorders. *The American Journal of Psychiatry*, *157*(5), 669–682. https://doi.org/10.1176/appi.ajp.157.5.669

Meneses, A., & Liy-Salmeron, G. (2012). Serotonin and emotion, learning and memory. *Reviews in the Neurosciences*, *23*(5–6), 543–553. https://doi.org/10.1515/revneuro-2012-0060

Messaoudi, M., Violle, N., Bisson, J. F., Desor, D., Javelot, H., & Rougeot, C. (2011). Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. *Gut Microbes*, *2*(4), 256–261. https://doi.org/10.4161/gmic.2.4.16108

Meyer, U., & Feldon, J. (2009). Prenatal exposure to infection: A primary mechanism for abnormal dopaminergic development in schizophrenia. *Psychopharmacology (Berl)*, *206*(4), 587–602. https://doi.org/10.1007/s00213-009-1504-9

Mika, A., Van Treuren, W., Gonzalez, A., Herrera, J. J., Knight, R., & Fleshner, M. (2015). Exercise is more effective at altering gut microbial composition and producing stable changes in lean mass in juvenile versus adult male f344 rats. *PLoS ONE*, *10*(5), e0125889. https://doi.org/10.1371/journal.pone.0125889

Miller, A. H., & Raison, C. L. (2016). The role of inflammation in depression: From evolutionary imperative to modern treatment target. *Nature Reviews Immunology*, *16*(1), 22–34. https://doi.org/10.1038/nri.2015.5

Mokhber, N., Shaghayegh, H., Talebi, M., & Tavassoli, A. (2016). Comparison of levels of depression in patients with excessive obesity before and after gastric bypass surgery. *Journal of Minimally Invasive Surgical Sciences*, *5*(4), e34947. https://doi.org/10.17795/minsurgery-34947

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The microbiome: Stress, health and disease. *Mammalian Genome*, *25*(1–2), 49–74. https://doi.org/10.1007/s00335-013-9488-5

Monda, V., Villano, I., Messina, A., Valenzano, A., Esposito, T., Moscatelli, F., … Messina, G. (2017). Exercise modifies the gut microbiota with positive health effects. *Oxidative Medicine and Cellular Longevity*, *2017*, 8. https://doi.org/10.1155/2017/3831972

Moret, C., & Briley, M. (2011). The importance of norepinephrine in depression. *Neuropsychiatric Disease and Treatment*, *7*(Suppl 1), 9–13. https://doi.org/10.2147/NDT.S19619

Morrison, D. J., & Preston, T. (2016). Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes*, *7*(3), 189–200. https://doi.org/10.1080/19490976.2015.1134082

Mu, C., Yang, Y., & Zhu, W. (2016). Gut microbiota: the brain peacekeeper. *Frontiers in Microbiology*, *7*, 345. https://doi.org/10.3389/fmicb.2016.00345

Mueller, N. T., Bakacs, E., Combellick, J., Grigoryan, Z., & Dominguez-Bello, M. G. (2015). The infant microbiome development: Mom matters. *Trends in Molecular Medicine*, *21*(2), 109–117. https://doi.org/10.1016/j.molmed.2014.12.002

Mulak, A., & Bonaz, B. (2015). Brain-gut-microbiota axis in Parkinson's disease. *World Journal of Gastroenterology*, *21*(37), 10609–10620. https://doi.org/10.3748/wjg.v21.i37.10609

Müller, C. P., & Homberg, J. R. (2015). The role of serotonin in drug use and addiction. *Behavioral Brain Research*, *277*, 146–192. https://doi.org/10.1016/j.bbr.2014.04.007

Murphy, S. E., Yiend, J., Lester, K. J., Cowen, P. J., & Harmer, C. J. (2009). Short-term serotonergic but not noradrenergic antidepressant administration reduces attentional vigilance to threat in healthy volunteers. *International Journal of Neuropsychopharmacology*, *12*(2), 169–179. https://doi.org/10.1017/S1461145708009164

Myles, I. A. (2014). Fast food fever: Reviewing the impacts of the Western diet on immunity. *Nutrition Journal*, *13*, 61. https://doi.org/10.1186/1475-2891-13-61

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology and Motility*, *23*(3), 255–264, e119. https://doi.org/10.1111/j.1365-2982.2010.01620.x

Niccoli, T., & Partridge, L. (2012). Ageing as a risk factor for disease. *Current Biology*, *22*(17), R741–752. https://doi.org/10.1016/j.cub.2012.07.024

Noakes, T. D. (2012). Fatigue is a brain-derived emotion that regulates the exercise behavior to ensure the protection of whole body homeostasis. *Frontiers in Physiology*, *3*, 82. https://doi.org/10.3389/fphys.2012.00082

Ochner, C. N., Kwok, Y., Conceição, E., Pantazatos, S. P., Puma, L. M., Carnell, S., … Geliebter, A. (2011). Selective reduction in neural responses to high calorie foods following gastric bypass surgery. *Annals of Surgery*, *253*(3), 502–507. https://doi.org/10.1097/SLA.0b013e318203a289

Ochner, C. N., Stice, E., Hutchins, E., Afifi, L., Geliebter, A., Hirsch, J., & Teixeira, J. (2012). Relation between changes in neural responsivity and reductions in desire to eat high-calorie foods following gastric bypass surgery. *Neuroscience*, *209*, 128–135. https://doi.org/10.1016/j.neuroscience.2012.02.030

OECD (2017). *Health at a glance 2017: OECD indicators*. Paris, France: OECD Publishing.

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioral Brain Research*, *277*, 32–48. https://doi.org/10.1016/j.bbr.2014.07.027

Oriach, C. S., Robertson, R. C., Stanton, C., Cryan, J. F., & Dinan, T. G. (2016). Food for thought: The role of nutrition in the microbiota-gut–brain axis. *Clinical Nutrition Experimental*, *6*, 25–38. https://doi.org/10.1016/j.yclnex.2016.01.003

Ortiz, G. G., Benítez-King, G. A., Rosales-Corral, S. A., Pacheco-Moisés, F. P., & Velázquez-Brizuela, I. E. (2008). Cellular and biochemical actions of melatonin which protect against free radicals: role in neurodegenerative disorders. *Current Neuropharmacology*, *6*(3), 203–214. https://doi.org/10.2174/157015908785777201

Palmeira, A. L., Branco, T. L., Martins, S. C., Minderico, C. S., Silva, M. N., Vieira, P. N., … Teixeira, P. J. (2010). Change in body image and psychological well-being during behavioral obesity treatment: Associations with weight loss and maintenance. *Body Image*, *7*(3), 187–193. https://doi.org/10.1016/j.bodyim.2010.03.002

Palsson, O. S., & Whitehead, W. E. (2013). Psychological treatments in functional gastrointestinal disorders: A primer for the gastroenterologist. *Clinical Gastroenterology and Hepatology*, *11*(3), 208–216; quiz e222–203. https://doi.org/10.1016/j.cgh.2012.10.031

Patel, J. P., & Frey, B. N. (2015). Disruption in the blood-brain barrier: The missing link between brain and body inflammation in bipolar disorder? *Neural Plasticity*, *2015*, 12. https://doi.org/10.1155/2015/708306

Paulus, M. P., & Stein, M. B. (2010). Interoception in anxiety and depression. *Brain Structure and Function*, *214*(5–6), 451–463. https://doi.org/10.1007/s00429-010-0258-9

Peng, L., Li, Z. R., Green, R. S., Holzman, I. R., & Lin, J. (2009). Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *Journal of Nutrition*, *139*(9), 1619–1625. https://doi.org/10.3945/jn.109.104638

Polidano, C., Zhu, A., & Bornstein, J. C. (2017). The relation between caesarean birth and child cognitive development. *Scientific Reports*, *7*(11483), 1–10. https://doi.org/10.1038/s41598-017-10831-y

Poti, J. M., Mendez, M. A., Ng, S. W., & Popkin, B. M. (2015). Is the degree of food processing and convenience linked with the nutritional quality of foods purchased by US households? *American Journal of Clinical Nutrition*, *101*(6), 1251–1262. https://doi.org/10.3945/ajcn.114.100925

Quercia, S., Candela, M., Giuliani, C., Turroni, S., Luiselli, D., Rampelli, S., … Pirazzini, C. (2014). From lifetime to evolution: Timescales of human gut microbiota adaptation. *Frontiers in Microbiology*, *5*, 1–9. https://doi.org/10.3389/fmicb.2014.00587

Rao, A. V., Bested, A. C., Beaulne, T. M., Katzman, M. A., Iorio, C., Berardi, J. M., & Logan, A. C. (2009). A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathogens*, *1*(1), 6. https://doi.org/10.1186/1757-4749-1-6

Rea, K., Dinan, T. G., & Cryan, J. F. (2016). The microbiome: A key regulator of stress and neuroinflammation. *Neurobiol Stress*, *4*, 23–33. https://doi.org/10.1016/j.ynstr.2016.03.001

Rhee, S. H., Pothoulakis, C., & Mayer, E. A. (2009). Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature Reviews Gastroenterology & Hepatology*, *6*(5), 306–314. https://doi.org/10.1038/nrgastro.2009.35

Rogers, G. B., Keating, D. J., Young, R. L., Wong, M. L., Licinio, J., & Wesselingh, S. (2016). From gut dysbiosis to altered brain function and mental illness: Mechanisms and pathways. *Molecular Psychiatry*, *21*(6), 738–748. https://doi.org/10.1038/mp.2016.50

Roohafza, H., Bidaki, E. Z., Hasanzadeh-Keshteli, A., Daghaghzade, H., Afshar, H., & Adibi, P. (2016). Anxiety, depression and distress among irritable bowel syndrome and their subtypes: An epidemiological population based study. *Advanced Biomedical Research*, *5*, 183. https://doi.org/10.4103/2277-9175.190938

Rubio-Ruiz, M. E., Peredo-Escárcega, A. E., Cano-Martínez, A., & Guarner-Lans, V. (2015). An evolutionary perspective of nutrition and inflammation as mechanisms of cardiovascular disease. *International Journal of Evolutionary Biology*, *2015*, 1–10. https://doi.org/10.1155/2015/179791

Sampson, T. R., & Mazmanian, S. K. (2015). Control of brain development, function, and behavior by the microbiome. *Cell Host & Microbe*, *17*(5), 565–576. https://doi.org/10.1016/j.chom.2015.04.011

Sarwer, D. B., & Steffen, K. J. (2015). Quality of life, body image and sexual functioning in bariatric surgery patients. *European Eating Disorders Review*, *23*(6), 504–508. https://doi.org/10.1002/erv.2412

Scheperjans, F., Aho, V., Pereira, P. A. B., Koskinen, K., Paulin, L., Pekkonen, E., … Auvinen, P. (2015). Gut microbiota are related to Parkinson's disease and clinical phenotype. *Movement Disorders*, *30*(3), 350–358. https://doi.org/10.1002/mds.26069

Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., & Burnet, P. W. (2015). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology (Berl)*, *232*(10), 1793–1801. https://doi.org/10.1007/s00213-014-3810-0

Schnorr, S. L., Sankaranarayanan, K., Lewis, C. M. Jr, & Warinner, C. (2016). Insights into human evolution from ancient and contemporary microbiome studies. *Current Opinion in Genetics & Development*, *41*, 14–26. https://doi.org/10.1016/j.gde.2016.07.003

Schoknecht, K., & Shalev, H. (2012). Blood-brain barrier dysfunction in brain diseases: Clinical experience. *Epilepsia*, *53*(Suppl 6), 7–13. https://doi.org/10.1111/j.1528-1167.2012.03697.x

Sekirov, I., Russell, S. L., Antunes, L. C., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological Reviews*, *90*(3), 859–904. https://doi.org/10.1152/physrev.00045.2009

Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biology*, *14*(8), e1002533. https://doi.org/10.1371/journal.pbio.1002533

Sevelsted, A., Stokholm, J., Bonnelykke, K., & Bisgaard, H. (2015). Cesarean section and chronic immune disorders. *Pediatrics*, *135*(1), e92–e98. https://doi.org/10.1542/peds.2014-0596

Shahar, D., Shai, I., Vardi, H., Shahar, A., & Fraser, D. (2005). Diet and eating habits in high and low socioeconomic groups. *Nutrition*, *21*(5), 559–566. https://doi.org/10.1016/j.nut.2004.09.018

Sheedy, J. R., Wettenhall, R. E., Scanlon, D., Gooley, P. R., Lewis, D. P., McGregor, N., … De Meirleir, K. L. (2009). Increased d-lactic acid intestinal bacteria in patients with chronic fatigue syndrome. *In Vivo*, *23*(4), 621–628.

Sheflin, A., Melby, C. L., Carbonero, F., & Weir, T. L. (2017). Linking dietary patterns with gut microbial composition and function. *Gut Microbes*, *8*(2), 113–129. https://doi.org/10.1080/19490976.2016.1270809

Shi, Y., Dong, Y., Huang, W., Zhu, D., Mao, H., & Su, P. (2016). Fecal microbiota transplantation for ulcerative colitis: A systematic review and meta-analysis. *PLoS ONE*, *11*(6), e0157259. https://doi.org/10.1371/journal.pone.0157259

Shishov, V. A., Kirovskaya, T. A., Kudrin, V. S., & Oleskin, A. V. (2009). Amine neuromediators, their precursors, and oxidation products in the culture of *Escherichia coli* K-12. *Applied Biochemistry and Microbiology*, *45*(5), 494–497. https://doi.org/10.1134/S0003683809050068

Singh, A. R. (2010). Modern medicine: Towards prevention, cure, well-being and longevity. *Mens Sana Monographs*, *8*(1), 17–29. https://doi.org/10.4103/0973-1229.58817

Singh, R. K., Chang, H.-W., Yan, D. I., Lee, K. M., Ucmak, D., Wong, K., … Liao, W. (2017). Influence of diet on the gut microbiome and implications for human health. *Journal of Translational Medicine*, *15*(1), 73. https://doi.org/10.1186/s12967-017-1175-y

Sirin, S. R. (2005). Socioeconomic status and academic achievement: a meta-analytic review of research. *Review of Educational Research*, *75*(3), 417–453. https://doi.org/10.3102/00346543075003417

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., … Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*, *341*(6145), 569–573. https://doi.org/10.1126/science.1241165

Song, P., Patel, N. B., Gunther, S., Li, C.-S., Liu, Y., Lee, C. Y. G., … Wong, M. S. (2016). Body image and quality of life: changes with gastric bypass and body contouring. *Annals of Plastic Surgery*, *76*(Suppl 3), S216–S221. https://doi.org/10.1097/SAP.0000000000000788

Song, S. J., Lauber, C., Costello, E. K., Lozupone, C. A., Humphrey, G., Berg-Lyons, D., … Knight, R. (2013). Cohabiting family members share microbiota with one another and with their dogs. *eLife*, *2*, e00458. https://doi.org/10.7554/eLife.00458.

Srivastava, K., & Das, R. C. (2015). Personality and health: Road to well-being. *Industrial Psychiatry Journal*, *24*(1), 1–4. https://doi.org/10.4103/0972-6748.160905

Stanislawski, M. A., Dabelea, D., Wagner, B. D., Sontag, M. K., Lozupone, C. A., & Eggesbø, M. (2017). Pre-pregnancy weight, gestational weight gain, and the gut microbiota of mothers and their infants. *Microbiome*, *5*(1), 113. https://doi.org/10.1186/s40168-017-0332-0

Stasi, C., Rosselli, M., Zignego, A. L., Laffi, G., & Milani, S. (2014). Serotonin and its implication in the side-effects of interferon-based treatment of patients with chronic viral hepatitis: Pharmacological interventions. *Hepatology Research*, *44*(1), 9–16. https://doi.org/10.1111/hepr.12116

Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J. A., & Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, Behavior, and Immunity*, *48*, 258–264. https://doi.org/10.1016/j.bbi.2015.04.003

Sudo, N. (2012). Role of microbiome in regulating the HPA axis and its relevance to allergy. *Chemical Immunology and Allergy*, *98*, 163–175. https://doi.org/10.1159/000336510

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., … Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *Journal of Physiology*, *558*(Pt 1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Sun, J., Wang, F., Li, H., Zhang, H., Jin, J., Chen, W., … Liu, C. (2015). Neuroprotective effect of sodium butyrate against cerebral ischemia/reperfusion injury in mice. *BioMed Research International*, *2015*, 395895. https://doi.org/10.1155/2015/395895

Tamburini, S., Shen, N., Wu, H. C., & Clemente, J. C. (2016). The microbiome in early life: Implications for health outcomes. *Nature Medicine*, *22*(7), 713–722. https://doi.org/10.1038/nm.4142

Tana, C., Umesaki, Y., Imaoka, A., Handa, T., Kanazawa, M., & Fukudo, S. (2010). Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterology and Motility*, *22*(5), 512–519, e114–515. https://doi.org/10.1111/j.1365-2982.2009.01427.x

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., … Bordenstein, S. R. (2016). Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes. *mSystems*, *1*(2), e00028-16.https://doi.org/10.1128/mSystems.00028-16

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., … Mayer, E. A. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394–1401, 1401.e1391–1394. https://doi.org/10.1053/j.gastro.2013.02.043

Ussar, S., Fujisaka, S., & Kahn, C. R. (2016). Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. *Molecular Metabolism*, *5*(9), 795–803. https://doi.org/10.1016/j.molmet.2016.07.004

Uyeda, J. C., Hansen, T. F., Arnold, S. J., & Pienaar, J. (2011). The million-year wait for macroevolutionary bursts. *Proceedings of the National Academy of Sciences USA*, *108*(38), 15908–15913. https://doi.org/10.1073/pnas.1014503108

Van Oudenhove, L., Crowell, M. D., Drossman, D. A., Halpert, A. D., Keefer, L., Lackner, J. M., … Levy, R. L. (2016). Biopsychosocial aspects of functional gastrointestinal disorders. *Gastroenterology*, *150*(6), 1355–1367. https://doi.org/10.1053/j.gastro.2016.02.027

Vangay, P., Ward, T., Gerber, J. S., & Knights, D. (2015). Antibiotics, pediatric dysbiosis, and disease. *Cell Host & Microbe*, *17*(5), 553–564. https://doi.org/10.1016/j.chom.2015.04.006

Veatch, O. J., Goldman, S. E., Adkins, K. W., & Malow, B. A. (2015). Melatonin in children with autism spectrum disorders: How does the evidence fit together? *Journal of Nature and Science*, *1*(7), e125.

Voreades, N., Kozil, A., & Weir, T. L. (2014). Diet and the development of the human intestinal microbiome. *Frontiers in Microbiology*, *5*(494), 1–9. https://doi.org/10.3389/fmicb.2014.00494

Vozeh, S. (2003). Is the increasing use of evidence-based pharmacotherapy causing the renaissance of complementary medicine? *British Journal of Clinical Pharmacology*, *56*(3), 292–296. https://doi.org/10.1046/j.0306-5251.2003.01879.x

Wallis, A., Butt, H., Ball, M., Lewis, D. P., & Bruck, D. (2016). Support for the microgenderome: Associations in a human clinical population. *Scientific Reports*, *6*, 1–9. https://doi.org/10.1038/srep19171

Warren, R. P., & Singh, V. K. (1996). Elevated serotonin levels in autism: Association with the major histocompatibility complex. *Neuropsychobiology*, *34*(2), 72–75. https://doi.org/10.1159/000119295

Welly, R. J., Liu, T.-W., Zidon, T. M., Rowles, J. L., Park, Y.-M., Smith, T. N., … Vieira-Potter, V. J. (2016). Comparison of diet vs. exercise on metabolic function & gut microbiota in obese rats. *Medicine and Science in Sports and Exercise*, *48*(9), 1688–1698. https://doi.org/10.1249/MSS.0000000000000964

Whitaker-Azmitia, P. M. (2001). Serotonin and brain development: Role in human developmental diseases. *Brain Research Bulletin*, *56*(5), 479–485. https://doi.org/10.1016/S0361-9230(01)00615-3

Wong, R. K., Yang, C., Song, G.-H., Wong, J., & Ho, K.-Y. (2015). Melatonin regulation as a possible mechanism for probiotic (VSL#3) in irritable bowel syndrome: a randomized double-blinded placebo study. *Digestive Diseases and Sciences*, *60*(1), 186–194. https://doi.org/10.1007/s10620-014-3299-8

World Health Organisation (WHO) (2017). *Depression (Fact sheet No. 369)*. Retrieved from http://www.who.int/mediacentre/factsheets/fs369/en/

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., … Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, *334*(6052), 105–108. https://doi.org/10.1126/science.1208344

Yamamoto, K., & Hornykiewicz, O. (2004). Proposal for a noradrenaline hypothesis of schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *28*(5), 913–922. https://doi.org/10.1016/j.pnpbp.2004.05.033

Yamamura, S., Morishima, H., Kumano-go, T., Suganuma, N., Matsumoto, H., Adachi, H., … Takeda, M. (2009). The effect of *Lactobacillus helveticus* fermented milk on sleep and health perception in elderly subjects. *European Journal of Clinical Nutrition*, *63*(1), 100–105. https://doi.org/10.1038/sj.ejcn.1602898

Yarandi, S. S., Peterson, D. A., Treisman, G. J., Moran, T. H., & Pasricha, P. J. (2016). Modulatory effects of gut microbiota on the central nervous system: How gut could play a role in neuropsychiatric health and diseases. *Journal of Neurogastroenterology and Motility*, *22*(2), 201–212. https://doi.org/10.5056/jnm15146

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*(7402), 222–227. https://doi.org/10.1038/nature11053

Yoto, A., Murao, S., Motoki, M., Yokoyama, Y., Horie, N., Takeshima, K., … Yokogoshi, H. (2012). Oral intake of gamma-aminobutyric acid affects mood and activities of central nervous system during stressed condition induced by mental tasks. *Amino Acids*, *43*(3), 1331–1337. https://doi.org/10.1007/s00726-011-1206-6

Zagajewski, J., Drozdowicz, D., Brzozowska, I., Hubalewska-Mazgaj, M., Stelmaszynska, T., Laidler, P. M., & Brzozowski, T. (2012). Conversion L-tryptophan to melatonin in the gastrointestinal tract: The new high performance liquid chromatography method enabling simultaneous determination of six metabolites of L-tryptophan by native fluorescence and UV-VIS detection. *Journal of Physiology and Pharmacology*, *63*(6), 613–621.

Zhang, H., DiBaise, J. K., Zuccolo, A., Kudrna, D., Braidotti, M., Yu, Y., … Krajmalnik-Brown, R. (2009). Human gut microbiota in obesity and after gastric bypass. *Proceedings of the National Academy of Sciences USA*, *106*(7), 2365–2370. https://doi.org/10.1073/pnas.0812600106

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Chapter 3: Expanded Introduction

The human GM is a large, diverse, and complex ecosystem of microorganisms. It comprises of more than 100 trillion individual microorganisms belonging to various taxonomic classifications. The GM is comprised of bacteria, archaea, protozoa, helminths, viruses, and fungi (Jandhyala et al., 2015; Loke & Lim, 2015; Scarpellini et al., 2015). However, the vast majority of research to date has focused on the bacterial component of this ecosystem (Hillman et al., 2017). The GM demonstrates immense inter-individual diversity. While over 2000 species of GM have been identified (Almeida et al., 2019), it has been suggested that an individual's GM is comprised of between 100 and 500 species (King et al., 2019; Quigley, 2013). As such, specific combinations of microorganisms are essentially limitless. Demonstrating this diversity, the human genome is approximately 99.9% similar between individuals, whereas the gut microbiome can be up to 80 to 90% different between individuals (Turnbaugh et al., 2009; Ursell et al., 2012). Personal GM composition has even been described as unique as a fingerprint (Browne et al., 2016).

There is also a large amount of intra-site variability of microbiota composition within the gut. Each component of the GI tract represents an ecological niche which provides variations in microbial exposure, nutrient availability, microbial composition, and host immunological responses (Pereira & Berry, 2017). The exact principles which govern the structure of these highly complex microbial communities residing within the ecological niches along the GI tract remain poorly understood (Pereira & Berry, 2017), however the drivers are likely to be based in evolution and related to the specific tasks performed along each section of the GI tract. The mouth and the large intestine, or colon, are the most densely populated areas of the GI tract regarding colonisation of microorganisms (Hillman et al., 2017). Alternatively, the oesophagus, stomach, and duodenum are the least colonised areas of the GI tract (Hunt et al., 2015). As an example, factors that are believed to restrict bacterial growth in the small intestine are higher levels of acid, oxygen, and antimicrobials, together with shorter transit time in the small intestine compared to the larger intestine (Donaldson et al., 2016). This makes the small intestine a more hostile environment for bacterial growth.

3.1 Biological Terminology and Conventions

While this thesis is written from a psychology perspective for a psychology audience, it is necessarily multidisciplinary. As such, it is prudent to comment on specific terminology that is typically used in microbiological research, but may be less familiar to psychology researchers. Firstly, the term *microbiome* is often incorrectly used interchangeably with the term *microbiota*. While the term microbiota refers to a community of microorganisms, microbiome refers to the microorganisms, their genomes, and their surrounding environmental conditions (Marchesi & Ravel,

2015). The gut microbiome is characterised by approximately 3.3 million non-redundant microbial genes, which is about 150 times greater than the human genome (Qin et al., 2010). Methodological constraints mean that the current thesis is focused on the gut *microbiota* alone, without consideration of the broader microbiome.

Specifically, the current thesis will be investigating the relationship between GM and psychological symptom expression at the taxonomic rank of species. Taxonomic ranks are hierarchically organised biological classifications of organisms. Higher taxonomic ranks are broader and organisms within them are more genetically diverse, whereas organisms within the same lower taxonomic ranks have greater genetic similarity (Al Bander et al., 2020). Variability also increases at lower taxonomic ranks. At the phylum level, 12 phyla have been identified (Thursby & Juge, 2017) however the GM is predominated by *Firmicutes* and *Bacteroidetes* (Jandhyala et al, 2015), making up approximately 90% of this ecosystem (Rinninella et al., 2019). At the genus level, the *Firmicutes* phylum is represented by more than 200 different genera including *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminococcus*, while the *Bacteroidetes* phulym primarily consists of the genera *Bacteroides* and *Prevotella*. While less abundant, other phyla are also present within the gut, with the *Actinobacteria* phylum mainly represented by *Bifidobacterium*, *Proteobacteria* by *Escherichia*, *Fusobacteria* by *Fusobacterium*, and *Verrucomicrobia* by *Akkermansia* (Rinninella et al., 2019). At the species level, it is now widely accepted that there are over 2000 different species, and more impressively, over 7000 different strains residing in the gut alone (Almeida et al., 2019; Anglin et al., 2015). As such, this means there are virtually limitless possible combinations of microorganisms within an individual's GM, explaining the immense inter-individual differences in GM composition.

There are also specific conventions regarding the reporting of species level data in microbiology, outlined by the Centers for Disease Control and Prevention (CDC; 2014). When referring to a specific microorganism, the genus must always precede the name of the species. For example, if referring to the species *Bifidobacterium bifidum*, the genus (*Bifidobacterium*) must be stated first. Once the genus has been stated, the genus shorthand should be used all subsequent times that species is referred to. The genus shorthand is the first letter of the genus, capitalised and followed by a full stop, subsequently followed by the species name. In the case of *Bifidobacterium bifidum*, this would be reported as *B. bifidum*. The genus shorthand must not be used unless it has previously been stated, and it is followed by a specific species. Throughout the current thesis, this convention is not strictly adhered to given that the intended audience is one with a psychology background. A psychologist may not be expected to understand that the 'B' in this instance refers to

Bifidobacterium, and not to another genus such as *Bacteroides*. (A microbiologist would be expected to understand this based upon the species name that follows). As such, to aid understanding, on some occasions the full genus name is repeated.

3.2 Brain-Gut-Microbiota-Axis (BGMA)

The BGMA refers to the intimate relationship between the brain and the gut, while acknowledging the important moderating role of GM (e.g., Carabotti et al., 2015; Grenham et al., 2011; Kelly et al., 2016). Still referred to as a bidirectional relationship (Osadchiy et al., 2019), Rea et al. (2016) more accurately describe the BGMA as a multidirectional relationship between the brain and the gut. The conceptualisation of the BGMA as being a simple bidirectional relationship undersells the true complexity of this intricate communication system. It is a multidirectional relationship in the sense that each component of this communication network can moderate the function of other systems involved. Further exemplifying the multidirectional nature of this communication network, other axes involving GM have been conceptualised, such as the gut-liver axis (e.g., Albillos et al., 2020; Konturek et al., 2018). As such, it is possible that GM influence cognitive functioning and psychological symptom expression through their interaction and communication with other bodily systems. The liver is used as an example here as it is considered to play an important role in immune functioning (Gao, 2016), and liver dysfunction has been associated with cognitive decline (hepatic encephalopathy) and psychological symptom expression (Choi et al., 2021; Felipo, 2013; Huang et al., 2017).

The BGMA is increasingly being recognised as playing an important role in homeostasis (Mu et al., 2016; Rea et al., 2016). Communication between the brain and the gut is maintained via a complex network which includes the CNS, autonomic nervous system (ANS), enteric nervous system (ENS), hypothalamic pituitary adrenal (HPA) axis, neural, endocrine and immune systems (e.g., Carabotti et al., 2015; Cryan & Dinan, 2012; Mayer, 2011; Moloney et al., 2014; Rhee et al., 2009). Essentially, this network means that signals from the brain are able to influence the motor, sensory, and secretory functions of the gut while signals from the gut are able to influence brain development, biochemistry, function, and behaviour (e.g., Cryan & O'Mahony, 2011; Grenham et al., 2011; Marques et al., 2014). While the GM are believed to regulate the CNS through neural, endocrine, metabolic, and immune pathways (Wang & Kasper, 2014), the CNS has the ability to influence the motility and secretory functions of the gut, as well as regulating signalling molecules which may alter intestinal permeability (Carabotti et al., 2015; Grenham et al., 2011). This changes the environment in which the microbes live, therefore influencing the composition of this ecosystem. Stress is noted as being one of the main CNS instigators in altering gut function and,

consequently, the microbiota in human and animal models (e.g., Bailey et al., 2011; Moloney et al., 2014; Rhee et al., 2009).

The primary focus of BGMA research is to demonstrate the association between GM and psychological processes and symptom expression. Over the last 15 or so years, there has been a proliferation of research into the BGMA which has demonstrated relationships between GM composition and the expression of psychological symptoms or disorders (Cryan et al., 2019). In the face of the abundance of evidence, it cannot be denied that GM are, at least to some degree, involved in psychological processes (e.g., Chen et al., 2018; Naseribafrouei et al., 2014; Jiang et al., 2015; Jiang et al., 2018; Lai et al., 2021; Vogt et al., 2017).

3.2.1 The Microgenderome

Growing evidence supports the concept of the microgenderome which implies that sex hormones play a role in modulating GM, therefore resulting in sex-specific host-microbiota interactions (Clarke et al., 2013; Mulak et al., 2014; Wallis et al., 2016). Sex differences in microbiota composition have been demonstrated in both pre-clinical and human studies (Vemuri et al., 2019). However, there are also studies which have demonstrated that the microbial composition of the gut is in fact similar between males and females (Wallis et al., 2016). Wallis et al. (2016) found that even though GM composition was similar between the sexes, certain bacterial genera, specifically *Clostridium*, *Lactobacillus*, and *Streptococcus*, were associated with self-reported symptoms in a sex divergent manner. These sex-specific interactions may offer some insight into differing prevalence rates between males and females for various illnesses which have been associated with certain GM profiles such as autism, anxiety, and depression (Moloney et al., 2014).

The direct influence of sex hormones on GM is demonstrated by changes in the microbial composition in the mother's gut during pregnancy (Collando et al., 2008). These changes in GM are believed to be related to alterations in metabolic and immunological functions which support foetal growth and development (Koren et al., 2012; Kumar & Magon, 2012). The composition and activity of GM has also been found to be influenced by life stages characterised by hormonal changes such as puberty and menopause (Conlon & Bird, 2014; Markle et al., 2013). In a cross-sectional study, Yuan et al. (2020) suggest that sex differences in GM composition exist prior to puberty, however these differences become more significant at puberty, further pointing towards an interaction between GM and sex hormones.

Sexual divergence with regards to immune functioning is well established, with sex differences affecting both innate and adaptive immune responses (Capone et al., 2018). Males tend to be more susceptible to infectious diseases and experience more severe symptoms, while females

exhibit higher rates of autoimmune diseases (Angum et al., 2020; Ingersoll, 2017; van Lunzen & Altfeld, 2014). Oestrogens are believed to play a complex role in the development of illnesses associated with a dysregulated inflammatory response and have been implicated in intestinal barrier function and intestinal permeability (Mulak et al., 2014; Straub, 2007). Females also produce stronger immune reactions, which may further explain the increased prevalence and severity of pain symptoms in females (Straub, 2007). On the other hand, androgens have been shown to be protective against visceral pain as they act to decrease pro-inflammatory mediators (Bianchi, 2018; So & Savidge, 2021). As aforementioned, one of the primary roles of GM is the entrainment of the immune system from a very early age. Potentially, compositional and/or functional differences in GM between males and females at a young age may contribute to these differences in immune functioning. Martin et al. (2016) found that *Lactobacillus* is more commonly found in the female gut compared to males in a study of infants in their first six months of life. Specifically, they found that at birth males had higher total bacterial counts, while females were more frequently colonised by *L. ruminis, L. gasseri, and L. reuteri*. Using a murine model, Yurkovetskiy et al. (2013) found that female specific pathogen free (SPF) mice had a 1.3 to 1.4 times higher incidence of type 1 diabetes, however, this sex difference did not exist in germ free (GF) mice. While Fransen et al. (2017) found microbiota-independent sex differences in immunity, they suggest that these differences may select a sex-specific microbiota which then exacerbates differences in the male and female immune systems.

While receiving less attention in almost all areas of GM research, protozoan members of the GM also provide evidence for the microgenderome. Certain protozoa, such as *Dientamoeba fragilis*, have been reported as commonly detected in females, however there are some inconsistencies in the literature with other studies demonstrating no sex differences (e.g., Barratt et al., 2011; Clemente et al., 2020; Miguel et al., 2018). Another common protozoan, *Blastocystis*, has also been shown to have a different impact on males and females (Nourisson et al., 2014). This suggests that the relationship between GM and sex hormones is not a simple one. Rather, sex hormones, gut bacteria, and gut protozoa may interact and moderate the relationships between each other.

Presenting an alternate view of the microgenderome, which is typically thought of from the perspective of sex hormones influencing GM, observational research demonstrates that a relatively common protozoan parasite, *Toxoplasma gondii*, may play a role in determining an infant's biological sex. The male:female sex ratio in mothers who were positive for *Toxoplasma* has been found to be higher compared to mothers who did not carry the protozoan (Flegr & Kaňková, 2020; Kaňková et al., 2007). Additionally, the probability of having a male child increased with increasing

anti-*Toxoplasma* antibodies. Flegr (2013) suggests that the increased probability of *Toxoplasma* infected mothers giving birth to male offspring may be related to the association between Toxoplasmosis and immunosuppression. Another possible explanation is the increased serum testosterone levels in those with *T.gondii* antibodies compared to those without. These results have since been replicated by Dama et al. (2016) and Shojaee et al. (2018). While these authors suggest that *T. gondii* may be one of the most important environmental factors influencing offspring sex ratio in humans, there remains a distinct lack of research. By inference, these findings also support the contention that maternal GM may affect the foetus in utero.

3.3 An Evolutionary Context for the Relationship Between the Gut and the Brain

Humans and their enteric microbiota have co-evolved in such a way that they share a mutualistic relationship where each rely heavily on one another for survival (Chow et al., 2010). Moeller et al. (2014) demonstrate that the human GM has undergone a significant transformation since the human-chimpanzee split. This evolutionary period was accompanied by a major shift from a mainly herbivorous diet to an omnivorous one, with an increase in animal consumption (Amato et al., 2015). The concomitant changes in GM which followed served functional purposes for host nutrition by salvaging energy that would otherwise be indigestible (Chow et al., 2010). For example, humans (compared to their non-human primate ancestors) have an increased abundance of *Bacteroides* which are associated with diets rich in protein and animal fat, and a decreased abundance of *Fibrobacter* which are involved in the fermentation of plants (Moeller et al. 2014). This change to a high quality (easily digestible and rich in nutrients), low carbohydrate and high protein diet was rich in iron, retinol, zinc, vitamin B12, and unsaturated fatty acids which provided the fuel for encephalisation (Rubio-Ruiz et al., 2015; Ruiz-Nunez et al., 2013). Clear distinctions in the composition and diversity of the human microbiota compared to that of other animals, including non-human primate ancestors, were also noted by Ley et al. (2008). GM have since been associated with the production (either directly or through indirect regulation) of brain-derived neurotrophic factor (BDNF), gamma aminobutyric acid (GABA) and other important neurotransmitters, as well as the development of the CNS (Gareau et al., 2011; Lyte, 2011; Sudo et al., 2004). It is therefore not unreasonable to suggest that the changing composition of GM played a key role in the increased size and complexity of the human brain during this period. While a high-quality diet provided the fuel for encephalisation, its easy digestibility also allowed for a reduction in the size of the human GI tract (Aiello, 1997). The brain (with a mass-specific metabolic rate of more than 22 times that of skeletal muscle) and the GI tract are two of the most metabolically expensive organs (along with the heart, liver, and kidneys). According to the Expensive Tissue Hypothesis, a reduction in the size of other metabolically expensive organs (primarily the gut) allows for a relatively large brain, without an

increase to the basal metabolic rate (Huang et al., 2018). This provides evidence of an early and extremely important link between the brain and the gut in human evolution.

During the Palaeolithic and Mesolithic eras, humans lived a hunter-gatherer lifestyle in nomadic societies of between 25 to 500 people which remained socially and culturally stable over thousands of generations, providing an environment of evolutionary adaptiveness (EEA; Higgs & Jones, 1999). Even when considering seasonal fluctuations in diet and lifestyle (Fragiadakis et al., 2019; Smits et al., 2017), these societies were characterised by high levels of physical activity, and high-quality diets consisting of minimally processed wild plants and animals (Broussard & Devkota, 2016). Also characteristic of these eras, however, was infection and famine which have been the leading causes of death for most of human history (Rubio-Ruiz et al., 2015). As a result of the EEA, human evolution had the time to select for a 'thrifty' genotype, along with an enhanced inflammatory function to combat these common causes of mortality. A thrifty genotype allowed for excess food (when available) to be stored as fat, which would then be of benefit during periods of food scarcity. It is also likely that insulin resistance was selected during this evolutionary period to prevent hypoglycaemia to the brain so as to preserve brain function during periods of famine (Rubo-Ruiz et al., 2015). Additionally, as human ancestors faced a higher infectious load compared to modern humankind, there were also evolutionary pressures for selection of an enhanced inflammatory function and immune response to protect against infectious disease. Inflammation results in increased sickness behaviours such as malaise, reduced appetite, loss of interest in physical and social activities, fragmented sleep, and fatigue (Dantzer et al., 2008; Moieni & Eisenberger, 2018). These behaviours are argued to be adaptive as they force the body into a state of energy conservation allowing for recuperation (Almond, 2013). Inflammation becomes problematic when it instead becomes low-grade and chronic in nature, as this essentially forces the body to be in a constant battle against a perceived threat resulting in allostatic overload (Liu et al., 2017), a concept discussed in Paper 1. Evidence suggests that this has implications for cognitive and psychological health (Minihane et al., 2015; Walker et al., 2014).

The introduction of agriculture and animal husbandry approximately 10,000 years ago significantly impacted on human diet and lifestyle. More recently, the industrial revolution further exacerbated these lifestyle and dietary changes (Ruiz-Nunez et al., 2013). Dietary changes included a significant increase in highly processed cereals rich in carbohydrates, refined sugars, sodium, omega-6, and trans-fatty acids. On the other hand, potassium, complex carbohydrates, fibre, omega-3, and unsaturated fatty acids were considerably reduced (Rubo-Ruiz et al., 2015). These changes are reflective of what is today termed the "Western diet" (Kopp, 2019; Statovci et al., 2017). Lifestyle

changes which accompanied the industrial revolution included an increase in indoor activities, more permanent residences, and sedentary behaviours. In addition to diet, physical environments are important in shaping an individual's microbiota as there are substantial differences between indoor dwelling microbes and external microbes found in air, water, and soil to which human ancestors were exposed (Broussard & Devkota, 2016). Furthermore, artificial lighting, jet-lag, and shift work have resulted in irregular sleep patterns causing alterations to the biological circadian rhythm which have been associated with unfavourable changes in GM (Benedict et al., 2016; Broussard & Devkota, 2015).

Increased public health measures were also implemented following the industrial revolution (Okada et al., 2010). These measures include decontamination of water supplies, pasteurisation and sterilisation of milk and other foods, vaccination against common childhood infections, and widespread antibiotic use following the advent of penicillin in 1928 (Okada et al., 2010). While these measures, along with other medical advancements, have undoubtedly increased life expectancy, eradicated deaths caused by common infections, and reduced the overall infectious load faced by modern humans, it appears to have come at the expense of an increase in sub-clinical, unhealthy states characterised chronic by low-grade inflammation (Broussard & Devkota, 2016; Ruiz-Nunez et al., 2013). According to the hygiene hypothesis, decreased exposure to microorganisms and infections in industrialised countries with good public health standards are at the root of increasing allergic, autoimmune, and inflammatory diseases (Okada et al., 2016). Certain illnesses are also considered to be an 'evolutionary accident' occurring as a result of increased longevity.

On an evolutionary timeline, these dietary and lifestyle changes are far too recent for biological selection to have had an impact on human evolution. As such, humans currently live in a period where their biological evolution and social environment are mismatched (Higgs & Jones, 1999). The previously optimal and protective thrifty genotype and enhanced inflammatory function persist, but are now in discordance with today's overabundance of processed high fat and high carbohydrate foods, sterile manufactured environments, and increasingly sedentary lifestyle (Broussard & Devkota, 2016). As such, humans in industrialised countries are living in an environment to which they have not adaptively evolved (Gluckman et al., 2011). Somewhat paradoxically then, previously adaptive genotypes may contribute to the increasing prevalence of "modern Western diseases" such as obesity, diabetes, inflammatory bowel disease, cardiovascular disease, allergies, and some cancers (e.g., Omenn, 2010; Ruiz-Nunez et al., 2013). In 2019, heart disease was by far the leading cause of death globally, with diabetes also in the top 10 (World Health Organisation [WHO], 2020). Despite these global figures, there is evidence to suggest that modern

Western diseases are rare or non-existent in more traditional hunter-gather societies such as Australian aboriginal populations in remote regions of Northern Australia and Amazonian, Malawian, and Tanzanian tribes (e.g., Broussard & Devkota, 2016; Gurven et al., 2009; O'Dea, 1991; Omenn, 2010; Schnorr et al., 2014). Comparative investigations reveal that although modern day hunter-gather populations show a reduced microbial diversity compared to wild ancestors, these reductions are more profound in industrialised countries such as the United States of America (Moeller et al., 2014). This suggests that GM composition and diversity play a role in the development of these diseases, with increased diversity generally associated with health (Moloney et al., 2014; Mosca et al., 2016).

Microbes, on the other hand, evolve much more rapidly than their human host (Bliven & Maurelli, 2016). Microbes have an innate plasticity which allows them to quickly adapt to both environmental and internal states in order to maintain a mutualistic health promoting relationship with their human host (Quercia et al., 2014). Wu et al. (2011) suggest that short- and long-term dietary changes impact different microbial groups, with long term diet being associated with compositional divisions seen between modern and ancestral humans as well as different cultural groups. Increasing consumption of refined sugars and calorie-dense foods challenge the adaptive abilities of the human GM. Consumption of these foods results in adaptive changes to the GM (such as decreased diversity and richness) which diverge from the mutualistic relationship with their human host and can lead to disease (Singh et al., 2017; Zinöcker & Lindseth, 2018). In particular, a continued loss of fibre from the modern Western diet will lead to continual depletion of short-chain fatty acids (SCFAs) which are an essential component of maintaining gut health and functionality (e.g., Blaak et al., 2020; den Besten et al., 2013). When a Western style diet is sustained over consecutive generations, less diverse microbiotas are genetically passed down through the process of vertical transmission from mother to infant (Sonnenberg et al., 2016). Sonnenberg et al. (2016) demonstrated, using a murine model, that GM diversity can be restored by the timely re-introduction of dietary fibre, however, after four generations, the re-introduction of dietary fibre is unable to restore lost microbial species. It is suggested that the sharp increase in various disease states, over the last 50-100 years in particular, may in part be due to genetically inherited less diverse GM which are under evolutionary pressure to shift from a mutualistic relationship with their human host, to a more antagonistic one (Broussard & Devkota, 2016; Quercia et al., 2014).

3.4 Gut Microbiota as an Essential and Inseparable Part of Human Physiology

Microbes were on Earth for millions of years before human beings inhabited the planet. As such, there has never been a point in all of human history where we have lived without the microorganisms that reside within the gut. The gut is considered to be one of the most densely

populated ecosystems on Earth, let alone the human body itself (Kelsen & Wu, 2012; Rinninella et al., 2019). While it has often been reported that bacterial cells outnumber total human body cells by a ratio of 10:1 (e.g., Fujimura et al., 2010), this estimate has since been revised to approximately 1:1 (Sender et al., 2016). Even considering this revised estimate of 1:1 bacterial to human cells, the importance of microorganisms to human physiology is conspicuously obvious, making them an intrinsic part of human physiology, since at least an equal part of the genetic makeup of a human comes from their bacteria. Additionally, Sender et al. (2016) refer specifically to bacterial counts, as such, this does not account for the other microorganisms (such as protozoa and fungi) that also constitute microbial ecosystems.

Demonstrating its importance to human physiology, the GM has been referred to as a vital organ (e.g., Baquero & Nombela, 2012; Evans et al., 2013; Turroni et al., 2020). More specifically, Clarke et al. (2014) describe the GM as resembling an endocrine organ due to its ability to produce a large variety of metabolic and hormonal products that work at both the local intestinal level, but also have far reaching distal effects. While the GM does not physically resemble an organ, it does meet a number of classical criteria required for consideration as an organ, such as its ability to influence, and be influenced by, other organs (Lyte, 2010; O'Callaghan et al., 2016). Mentioned in Paper 1, faecal microbiota transplant (FMT) is a treatment that continues to grow in popularity (Grigoryan et al., 2020). FMT is the transplantation, or transferral, of a solution of faecal matter from a 'healthy' donor into the intestinal tract of a 'dysbiotic' recipient (Gupta et al., 2016). It is typically used for the eradication of *Clostridium difficile*, and in other gastrointestinal disorders (Hui et al., 2019; Rossen et al., 2015). In itself, the terminology regarding 'transplantation' of GM is in line with terminology used in regards to the transplantation of other organs.

Expanding on this idea that the GM is an organ is the fact that, just like other organs, the GM appears to be innate. It has been a long held belief that the human foetus is sterile (free of microbial inhabitants) and that microbial colonisation begins at birth (Milani et al., 2017). However, evidence suggests that microbial colonisation may instead begin in utero with the detection of microorganisms in amniotic fluid (DiGuilio et al., 2008; Stinson et al., 2019), the umbilical cord blood (Jiménez et al., 2005), placenta (Aagaard et al., 2014, Zhu et al., 2018), and meconium (Hu et al., 2019). Changes in gene expression in foetal intestines and placenta have also been noted in mothers receiving probiotic supplementation (Rautava et al., 2012). While it may seem unimportant to know whether colonisation begins in utero or from birth, this may have important implications as exposure to bacteria during gestation may impact on foetal development (Stinson et al., 2019). These implications may include in utero immune system entrainment, as well as gut and brain

development (Stinson et al., 2019). However, the idea that colonisation begins in utero remains highly controversial. Studies which have demonstrated the presence of microbes in placental tissue are criticised, primarily on the basis of contamination issues during testing (Blaser et al., 2021). As such, the evidence supporting the idea of in utero colonisation is, currently, not considered to be strong, but does warrant further investigation (Hornef & Penders, 2017; Perez-Muñoz et al., 2017). Nonetheless, just like other organs, the GM develops and matures as an individual ages and may impact on host aging processes (Derrien et al., 2019; Seidel & Valenzano, 2018; Vemuri et al., 2018).

Viewing the GM as a vital organ and an innate and inseparable part of a human being is in line with the concept of the "holobiont". The term holobiont refers to an individual host and its microbial community as a single, unified "superorganism" (Salvucci, 2019; Simon et al., 2019; Theis et al., 2016; van de Guchte et al., 2018). While this term theoretically also applies to other ecological communities (such as skin and vaginal microbiota), the current thesis focuses specifically on *gut* microbiota. Considering GM to be a part human anatomy also makes them a part of an individual's personal identity. Considering the GM to be part of an individual's personal identity places it squarely in the realm of psychological inquiry. This is demonstrated by a number of factors such as the co-evolution between the human host and GM, the immense interpersonal differences in GM composition (Lloyd-Price et al., 2016; Turnbaugh et al., 2009; Ursell et al., 2012), the link between GM and symptom expression (e.g., Jiang et al., 2015; Taylor et al., 2019; Wallis et al., 2016), and associations between GM and personality traits (e.g., Flegr, 2013; Johnson, 2020; Kim et al., 2018). All of these factors (e.g., interpersonal differences, psychological symptom expression, and personality) are the targets of enquiry for the discipline of psychology. As such, to understand an individual and what makes them who they are, and how they are presenting, there needs to be an appreciation of the involvement of their GM.

3.4.1 The Role of the Gut Microbiota

Three essential functions of GM are to: 1. protect against pathogen colonisation of the gut, 2. strengthen and maintain the intestinal epithelial barrier, and 3. absorb nutrients through metabolism (Wang & Kasper, 2014). Therefore, they play an essential role in maintaining homeostasis. GM also play an important role in the development and function of the ENS, CNS, HPA axis, and host immune system (e.g., Belkaid & Hand, 2014; Carabotti et al., 2015; Collins et al., 2014; Sudo et al., 2004; Vagnerová et al., 2019; Wang et al., 2018; Zheng et al., 2020).

Studies using germ-free (GF) mice demonstrate the importance of early colonisation with a diverse microbial community in the development of key neurological and physiological systems such as the CNS and ENS. GF mice, compared to their colonised specific pathogen free (SPF) counterparts

(those that are colonised but are free of known pathogens), exhibit dysregulated expression of neurotransmitters, poorer immune function, abnormalities in gut motility, differences in stress response and anxiety like behaviours, as well as cognitive and social deficits (Carabotti et al., 2015; Cryan & O'Mahony, 2011; Desbonnet et al., 2014; Grenham et al., 2011; Moloney et al., 2014). During this period, GM are believed to play an important role in the development of the ENS and CNS as well as the development and function of the HPA axis (e.g., Carabotti et al., 2015; Collins et al., 2014; Sudo et al., 2004). Early colonization of the gut also plays a vital role in establishing immunological and metabolic pathways (Wang et al., 2016).

3.4.2 Maintaining Homeostasis

Homeostasis of the gut ecosystem is achieved through mechanisms of communication and competition which leads to some species occurring in high abundance, while others occur in much lower abundance (Arumugam et al., 2011). Microbes in the gut must cooperate and share limited resources (space and nutrients) to promote stable coexistence and ecological diversity (Allen & Nowak, 2013). However, these microorganisms are under selective pressure to ensure their own fitness and survival, and must therefore compete for these resources (Hibbing et al., 2010). Microbes express various phenotypes when competing with their proximal neighbours through either exploitative (passive) or interference (active) methods (Ghoul & Mitri, 2016). One exploitative technique involves the consumption of a limiting resource (such as nutrients or space) thereby restricting its competitor's access to it. For example, a microbe may restrict a competitor's access to nutrients through the secretion of digestive enzymes which break down complex nutrient molecules or siderophores which access insoluble iron (Bauer et al., 2018; Griffin et al., 2004). However, production of these molecules is costly, and may benefit microbes which exploit these products, a competitive strategy referred to as cheating (Diggle et al., 2007; Ghoul & Mitri, 2016). Additionally, a microbe can restrict nutrients from its competitors by altering its own metabolic activities to allow for faster absorption of nutrients than its competitors (Bauer et al, 2018; Ghoul & Mitri, 2016). An example of interference competition is when a microbe produces antimicrobial agents such as bacteriocins, which target specific strains, or peptides which have a broader spectrum (Bauer et al., 2018).

Knowledge regarding communication between GM must be considered in light of the dynamic nature of their interactions, meaning that they can vary across conditions, space, and time (Coyte & Rakoff-Nahoum, 2019). As such, current knowledge of the precise interactions between GM remains limited. Additionally, as with most areas of research concerning the GM, the focus has been on bacterial communication, and much less is known about the influence of other members of

the gut including protozoa, viruses, and fungi (Coyte & Rakoff-Nahoum, 2019; Shkoporov & Hill, 2019).

3.5 Symbiosis and Dysbiosis

The delicately balanced ecosystem of the highly diverse microorganisms is essential in defining health and disease (Sekirov et al., 2010). Health is promoted when the GM is comprised of a diverse range of microorganisms in proportionate balance (microbiota symbiosis; Moloney et al., 2014). Alternatively, both physiological and psychological disease states are associated with imbalances in the composition of the microbiota (dysbiosis) caused by the introduction of pathogenic bacteria, or the over- or under-abundance of specific GM resulting in reduced overall diversity (Blumstein et al., 2014). A number of psychological symptoms and disorders have been associated with GM dysbiosis, some of which are summarised in Paper 3.

What exact composition constitutes a healthy GM has not been established and is complicated by the complexity of the microbiota itself, but also intra- and inter person variation exacerbated by geographical location, personal experiences, sex, and age (Lozupone et al., 2012; Rinninella et al., 2019). Therefore, perhaps an appropriate conceptualisation of a healthy GM is one that supports the activities for optimum systemic functioning (Bäckhed et al., 2012). What is generally described as a healthy GM composition is one that is diverse and has an approximate balance of microorganisms. However, there are cases in which important microbes have a relative abundance of less than 5%, but the ecosystem still functions.

In a state of symbiosis, the host-microbiota interrelationship is considered to be a mutualistic one where both the host and the GM benefit from one another. Having co-evolved together, the human GI tract provides GM with resources such as a suitable environment for colonisation, and nutrients coming predominantly from ingested food. Cooperation and competition between microbial inhabitants of the gut maintain a balanced community. In return, this microbial community performs key functions essential in defining health and disease (Sekirov et al., 2010; Wang & Kasper, 2014). In addition, the concept of functional redundancy means that a portion of the GM may inhabit the gut without providing the host with any specific benefit. In this case, the relationship between host and GM is also referred to as a commensal one.

Alternatively, a state of dysbiosis refers to quantitative or functional changes in the GM (Iacob & Iacob, 2019). Dysbiosis can be diet (Brown et al., 2012) or antibiotic (Feng et al., 2019) induced, but may also be due to other factors which influence the homeostatic mechanisms that control microbial populations (Dukowicz et al., 2007). In a state of dysbiosis, the symbiotic or commensal relationship between the host and gut microbes is impaired due to alterations in the

balance of resident microbiota, typically due to a reduced abundance of commensal organisms and an overgrowth of pathogenic organisms (McDonald et al., 2016). Typically, commensal organisms can also quickly become pathogenic in response to changes in their environment, or when the GI epithelial barrier is disrupted (Miskinyte et al., 2013). Small intestinal bacterial overgrowth (SIBO) is an example of dysbiosis where there is an overabundance of bacteria in the typically less densely populated small intestine, and is believed to be a result of changes in factors controlling bacterial growth such as the level of gastric acid and small intestine mobility (Dukowicz et al., 2007). Structural abnormalities of the GI tract and immune functioning are also believed to predispose individuals to SIBO (Lappinga et al., 2010; Pignata et al., 1990). With regards to psychological symptom expression, SIBO has been associated with anxiety and depression (Addolorato et al., 2008), and brain fog which refers to symptoms of mental confusion, impaired judgement, poor short-term memory, and difficulty concentrating (Rao et al., 2018).

Given that the delicately balanced ecosystem of the GM is not only comprised of bacteria, but is also home to protozoa, fungi, and viruses, it is important that these also be considered in discussions and research regarding symbiosis and dysbiosis. Given that the retrospective data used in the current thesis did not include data regarding viruses, viruses will not be further discussed herein. Compared to current knowledge regarding bacterial balance/imbalance, far less is known about the influence of the neglected constituents of the GM such as protozoa and fungi. A review by Iliev and Leonardi (2017) is one of few that draws attention to the role of fungi in shaping and maintaining host homeostasis, primarily through their influence on immune functioning. It may be the case that because the main focus of research in the past has been on the pathogenic potential of fungi, that understanding and recognition of their commensalism is lacking (Romo & Kumamoto, 2020). In a similar way, the impact of protozoan members of the gut is also poorly understood (Loke & Lim, 2016). Nomenclature in the literature demonstrates this skewed view of protozoan gut inhabitants which are often misidentified as parasites, by definition implying that they live at the expense of their host, which is in contradiction to the concept of symbiosis. Comparatively few studies have shed light on the potential commensalism or benefit of protozoa (Loke & Lim, 2016; Lukeš et al., 2015).

3.5.1 Altering the GM

An individual's GM is influenced by numerous factors including birth mode (vaginal or caesarean), full term or preterm delivery, feeding method (breast or bottle), introduction of solid foods, antibiotic use (of mother and infant), biological sex, and genetics (Blekhman et al., 2015; Busi et al., 2021; Henderickx et al., 2019; Homann et al., 2021; Ma et al., 2020; Martin et al., 2016; Mueller et al., 2015). Recent evidence also suggests that mothers' mood has a significant effect on

meconium, with children from mothers experiencing greater pregnancy related anxiety having a less diverse meconium, and a lower abundance of the *Enterococcaceae* family (Hu et al., 2019). Early life microbial colonisation is believed to have implications on health outcomes later in life (Sarkar et al., 2021). This may be because the establishment of the microbiota coincides with infant development, and the development and maturation of the CNS and immune system (Cryan et al., 2019; Gensollen et al., 2016).

In addition to GM being vertically transferred from mother to infant, they are also horizontally transmitted, being acquired from another person, animal, or the environment (Browne et al., 2017; Jiang et al., 2019; Trinh et al., 2018; Wang & Lin, 2021). Once established, a number of factors can still influence GM composition. These include a person's age, hormones, diet, pre- and probiotic use, antibiotic use, medications, environment, geolocation, culture, and numerous other lifestyle factors (e.g., Ahn & Hayes, 2021; De Filippo et al., 2010; Hasan & Yang, 2019; Leeming et al., 2019; Odamaki et al., 2016; Senghor et al., 2018; Vich Vila et al., 2020; Yoon & Kim, 2021). Having siblings or pets in the home has also been demonstrated to influence the composition of an individual's GM (e.g., Kates et al., 2020; Laursen et al., 2015; Tun et al., 2017). Interpersonal relationships have also been demonstrated to influence GM composition, with a recent study suggesting that the GM may present as a biological link between relationships and health (Dill-McFarland et al., 2019). This study found that relationships, especially close marital relationships, have a positive influence GM composition. This demonstrates a potential moderating role of GM in the association between social relationships and health that have long been demonstrated in sociological and psychological research (e.g., Teo et al., 2013; Umberson et al., 2010; Yang et al., 2016).

Studies involving modulation of GM through probiotic supplementation have demonstrated the potential to reduce psychological symptom expression (e.g., Benton et al., 2007; Chao et al., 2020; Kazemi et al., 2019; Lew et al., 2019; Messaoudi et al., 2011; Schmidt et al., 2015; Steenbergen et al., 2015; Tillisch et al., 2013; Wallis et al., 2018; Yamamura et al., 2009). Rao et al. (2018) also demonstrated that antibiotic administration can improve cognitive functioning in the case of SIBO. Additionally, dietary improvement has been associated with a reduction in depressive symptoms (Firth et al., 2019; Jacka et al., 2017) and Mediterranean diets tend to demonstrate a positive impact on cognitive functioning (Klimova et al., 2020; Loughrey et al., 2017). Evidence regarding the efficacy of FMT in reducing psychological symptom expression is less abundant, however it is suggested that it may be a feasible treatment option (Collyer et al., 2020; Fond et al., 2020; Meyyappan et al., 2020). However, further clinical trials would need to be conducted before any solid conclusions can

be drawn. Taken together, this evidence suggests that modulation of GM may be efficacious in reducing psychological symptom expression.

3.6 Potential Mechanisms of Action

While the precise mechanisms of action as to how GM effect psychological functioning are yet to be confirmed (Almeida et al., 2020; Radjabzadeh et al., 2020), four main pathways have been proposed. The mechanisms of action that have been proposed include neuronal, endocrine, immune, and metabolic pathways which are discussed below. There is considerable overlap between these pathways given that the metabolism of SCFAs influences activation of the vagus nerve, and is also essential for immune functioning (e.g., Silva et al., 2020; Venegas et al., 2019).

3.6.1 Neuronal Pathway

Lining the GI tract from the oesophagus to the rectum is the enteric nervous system (ENS; Avetisyan et al., 2015; Mayer, 2011). Comprised of complex neuronal networks and glial cells, the ENS is similar to the brain in structure, size, complexity, neurochemical communication, and function which has led to it being termed 'the second brain' (Gershon, 1999; Mayer, 2011). The ENS is innervated by the vagus nerve (cranial nerve X), carrying both afferent and efferent signals allowing for a bidirectional flow of information between the ENS and reflex and command centres of the CNS (Bercik et al., 2011; Bravo et al., 2011; Coss-Adame & Rao, 2014). The ENS is able to autonomously regulate many processes in the gut independently of CNS input such as motility, secretion, and blood flow, which are essential for nutrient absorption and waste elimination (Avetisyan et al., 2015; Rao & Gershon, 2016). The ENS differentiates the GI tract from all other peripheral (outside of the CNS) organs (Furness et al., 2014) and is considered to be the third branch of the autonomic nervous system (ANS; Mayer, 2011).

The vagus nerve is thought to be the fastest and most direct communication route between GM and the brain (Fülling et al., 2019). Early evidence of a link between vagal function and psychological symptom expression in humans comes from reports of an increase in psychological disorders following ablation of the vagus nerve as part of gastrectomy (Browning & Houseworth, 1953; Whitlock, 1961). Alternatively, vagal nerve stimulation has been demonstrated to be an efficacious adjunct treatment for treatment-resistant depression (Aaronson et al., 2013; Aaronson et al., 2017; Berry et al., 2013; McAllister-Williams et al., 2020). Using murine models, Bercik et al. (2011) and Bravo et al. (2011) demonstrated that the anxiolytic and antidepressant effects of *Bifidobacterium logum* and *Lactobacillus rhamnosus* were dependent on an intact vagus nerve, demonstrating its crucial role in GM-brain communication.

While GM do not come into direct contact with afferent vagal fibers, GM signal the CNS via the vagal nerve through their metabolites (SCFAs), the release of hormones (serotonin), or through epithelial cells that relay luminal signals (Bonaz et al., 2018). As such, the vagus nerve plays a crucial role in the process of interoception, sensing microbial compounds or metabolites, and sending that information to the CNS (Bonaz et al., 2018).

3.6.2 Endocrine and Neurotransmitter Pathway

As previously discussed, the GM is considered to be an endocrine organ due to its ability to produce a myriad of hormonal products (Clarke et al., 2014; O'Callaghan et al., 2016). These hormonal products have the ability to alter the function of the gut, but can also enter the blood stream to effect the function of distal organ systems (O'Callaghan et al., 2016). The GM play an important regulatory role in the metabolism and concentration of essential amino acid tryptophan which is a precursor for several neurotransmitters (Bosi et al., 2020). Examples of certain microbial genera and species which have been associated with the synthesis of neurotransmitters are presented in Table 1 of Paper 1 within the current thesis. Further, Yano et al. (2015) argue that host-microbiota interactions play a fundamental role in serotonin related biological processes. In the gut, serotonin has an effect on motor, sensory, and secretory functions, while in the CNS it plays a role in motor control, circadian rhythm, body temperature, and cerebellar regulation (Kim & Camilleri, 2000; O'Mahony et al., 2015). The role of serotonin in behaviours such as visceral pain, appetite, addiction, emotion, memory, and stress response is also well documented (e.g., Halford & Blundell, 2000; Hood et al., 2006; Marks et al., 2009; Meneses & Liy-Salmeron, 2012; Müller & Homberg, 2015).

3.6.3 Immune Pathway

GM influence immune responses in various ways, both functional and dysfunctional. One such way is the translocation of GM from the intestinal lumen into the bloodstream as a result of increased intestinal permeability, known as 'leaky gut' (Mu et al., 2017; Nagpal & Yadav, 2017). Outside of the gut lumen, GM stimulate specific receptors (toll-like receptors) on circulating immune cells, triggering an inflammatory immune response (Wiertsema et al., 2021; Zheng et al., 2020). Within the gut, specific microorganisms can also stimulate the production of either pro-inflammatory or anti-inflammatory cytokines, which can either up- or down-regulate an inflammatory immune response (Al Bander et al., 2020). This is of particular relevance in understanding the aetiology of psychological disorders, with a growing consensus that inflammation is plays an important role (e.g., Miller, 2020; Vogelzangs et al., 2013; Zazula et al., 2021).

3.6.4 Metabolic Pathway

GM are essential for the fermentation of non-digestible dietary fibres and intestinal mucus (Valdes et al., 2018). SCFAs (acetic acid, butyric acid, and propionic acid in particular) are one of the main metabolites of GM (Carabotti et al., 2015; Smith et al., 2013). SCFAs are believed to be one of the main regulators of BGMA crosstalk (Silva et al., 2020; Stilling et al., 2016; van de Wouw et al., 2018). They are believed to influence the size and function of regulatory T cells which play a crucial role in regulating intestinal inflammation (Smith et al., 2013). Additionally, SCFAs butyrate, and to a lesser extent propionate, have been found to enhance intestinal barrier functioning through their regulation of the tight junction proteins (Ohata et al., 2005; Peng et al., 2009). Maintaining proper intestinal barrier function is important as impaired function can lead to a gastrointestinal and systemic immune response, triggering inflammation which results in symptom expression (e.g., Mu et al., 2017; Shin & Kim, 2018).

In addition to SCFAs, GM also produce a variety of nutrients such as B vitamins and vitamin K (Ramakrishna, 2013; Yoshii et al., 2019). B vitamins and vitamin K are gaining interest for their possible role in brain function and cognition (Alisi et al., 2019; Kennedy, 2016). The development of BDNF has also been implicated with GM through the use of animal models (Sudo et al., 2004). BDNF is an important protein which is involved in the growth and survival of neurons (Grenham et al., 2011), making it a vital protein for cognitive performance in both the short and long term (Miranda et al., 2019; Piepmeier & Etnier, 2015). Changes in GM composition have been reported to effect microbial metabolites such as BDNF, which can consequently contribute to changes in behaviour (Soto et al., 2018).

3.7 Rationale for the Current Thesis

Research into the BGMA has proliferated over recent years (Zhu et al., 2020) however, much of this research to date remains heavily skewed towards the bacterial component of the gut ecosystem alone. While bacteria make up the largest component of the GM, other microbial members of this ecosystem play an important role in composition and function (Enaud et al., 2018; Matijašić et al., 2020). For example, fungi have also been described as commensal gut microorganisms (Scanlan & Marchesi, 2008), transient colonisers (Hoffmann et al., 2013), and as potential opportunistic pathogens (Gouba & Drancourt, 2015). While there are studies that have focused on the influence of fungi on psychological symptom expression (Rucklidge, 2013), such studies are limited, and tend to focus specifically on a single genus (such as *Candida*) or species (such as *Candida albicans*). Currently, two fungal species are described as probiotics (*Saccharomyces cerevisiae* and *S. cerevisiae boulardii*), however Lara-Hildago et al. (2017) state that a range of other fungal species may have a probiotic potential. As such, Huseyin et al. (2017) call for greater research

into the fungal component of the GM in an effort to understand their contribution to human health and disease. The current thesis will address this gap in the literature by analysing several fungal species in addition to bacterial species, and their relationship with psychological symptom expression.

Additionally, there is also very limited information regarding the effect of intestinal protozoa on psychological symptoms. The majority of research investigating intestinal protozoa are centred around gastrointestinal symptoms, with little to no research focusing on the relationship between protozoa and psychological symptom expression. The relatively scarce research that does exist in this area has focused almost exclusively on *Toxoplama gondii* or known pathogenic protozoa (such as *Giardia*) which have demonstrated relationships between protozoa and psychological symptoms (e.g., Bak et al., 2018; Markovitz et al., 2015; Yolken et al., 2017). Research investigating the relationship between intestinal protozoa *Blastocystis* and *Dientamoeba fragilis*, both of which are considered to be common constituents of the human GM is distinctly lacking (Lepczyńska et al., 2017; Oliveira-Arbex et al., 2021). Given the potential influence of other intestinal protozoa (such as *T. gondii*) on psychological functioning and symptom expression, it is important to investigate the influence of these common intestinal protozoa. As such, the current thesis fills this gap in the literature and provides an important first step for future research.

In addition to investigating the relatively under-explored relationships between psychological symptom expression and fungi and protozoa, the current thesis also adds weight to a continually growing body of literature investigating the relationship between GM and psychological symptom expression. While there is an abundance of research that currently exists in this area, the inconsistencies between the results of these studies demonstrates the need for continued research. The current study is one of few large-scale studies which utilised a clinically diverse sample. Additionally, the current study investigated a large range of microbiota, with more than 450 microbial species identified. In doing so, the current thesis investigates the relationship between psychological symptom expression and microbial species which have to date received little to no attention in previous research (such as *Enteroccocus durans*, *Leuconostoc lactis* and many others).

Furthermore, the current thesis also contributes to research regarding the microgenderome. Research investigating the concept of the microgenderome is relatively limited. The current study contributes to this area of investigation by examining sex differences in the abundance of specific microbial species and intestinal protozoa. Furthermore, the current study also investigates the relationship between bacterial, fungal, and protozoan members of the GM with psychological symptom expression separately for males and females. It is anticipated that this will provide valuable

insight into the relationship between microbiota and symptom expression which can be later be translated into clinically useful applications in terms of personalised, sex-specific treatment via the GM.

The current thesis aims to position the BGMA as falling within the purview of psychologists. This endeavour necessitates a theoretical review, followed by observational findings relating psychological symptoms with members of the gut ecosystem (bacteria, fungi, and protozoa). The concept of the microgenderome is also explored by investigating these associations in a sex-specific manner. The specific aims and hypotheses of this thesis by publication are outlined in Paper 2 and Paper 3.

Chapter 4: General Methodology

4.1 Participants

The participants included in the studies presented within this thesis were taken from a large retrospectively collected database comprising of results from 9,812 faecal microbial analysis (FMA) samples. The data was collected by Bioscreen, a Melbourne based laboratory specialising in FMA, between January 2013 and June 2015. Prospective participants were referred to Bioscreen by their medical practitioner (e.g., general practitioner, gastroenterologist). Stool samples were submitted for analysis as part of the investigatory process for intestinal dysbiosis, however the specific diagnostic status of patients was not identified. As such, this thesis is based on cross-sectional data from a broad range of potentially clinical and non-clinical presentations. Following data screening and cleaning procedures, and ensuring that inclusion and exclusion criteria (discussed below) were satisfied, the final sample for the current study consisted of a total of 4610 participants. Demographic information describing the sample can be found in Table 1. Available information regarding participant demographics was limited, including only sex, age, postcode, and name of the referring health professional. The participants' postcode and name of their referring doctor were not considered to be relevant to the current study and were therefore not used.

Table 1.

Sample demographic information

Sex	*n* (%)	Age range (in years)	Mean (*SD*) age
Male	1143 (24.80)	18-87	43.49 (13.87)
Female	3467 (75.20)	18-86	42.96 (13.26)
Total	4610 (100.00)	18-87	43.09 (13.42)

As can be seen in Table 1, the age range and mean age of male and female participants was similar, and did not differ significantly. There was a stark contrast in the percentage of male and female participants, however, similar gender ratios of participation in research are not unusual and this is in line with previous research studies (e.g., Jackson et al., 2015; Jakobsdottir et al., 2013; Wallis et al., 2016). Lobato et al. (2014) suggest that several social influences may play a part in men and women's decision to participate in research.

The number of participants varied across the papers included in this thesis according to what was being studied. For example, only the Bioscreen patients who had been tested for intestinal

protozoa were included in Paper 2 (*n*= 979). In Paper 3, all patients were included, however the use of pairwise deletion meant that sample size varied per analysis (based on each microorganism).

Ethics approval for the current study was obtained from the Victoria University Human Research Ethics Committee (HRE16-071).

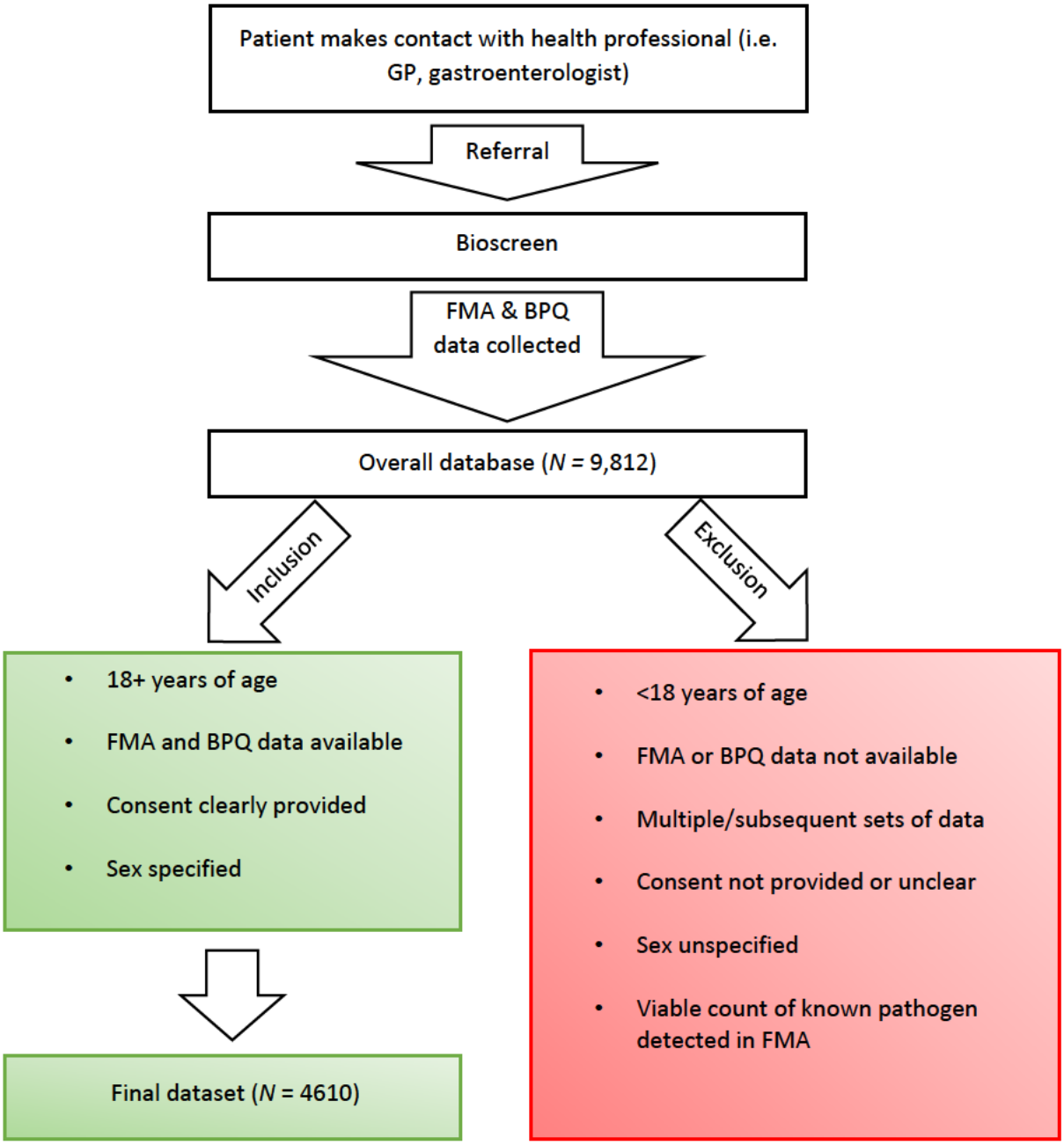
4.1.1 Inclusion and Exclusion Criteria

Inclusion criteria for the current study included participants needing to be 18 years of age or older. It was also a requirement for participation that each patient had available data from both their FMA and Bioscreen patient questionnaire (BPQ; a self-report measure of symptom severity). As the current study intended to investigate sex differences, patients were only included if they had specified their sex (male or female; no non-binary options were provided on the BPQ). Finally, it was also a requirement that eligible participants had provided consent for their information to be used for research purposes. As part of the BPQ, patients were asked to indicate (by ticking a check-box) whether they consented for their data to be used for research purposes. Only those who had checked 'yes' were included in the current study.

Bioscreen patients were excluded from participation in the current study if any of the aforementioned inclusion criteria were not met. For example, cases where information was only available for a patient's FMA or BPQ. Additionally, there were cases where patients had returned multiple times for FMAs and therefore had multiple sets of FMA and BPQ data. In these cases, only the earliest data available were utilised to ensure the basic assumption of independence of observation. Additionally, it was unclear whether patients had undergone some form of intervention (e.g., taken a probiotic or antibiotic) in the interim between their subsequent FMAs. Therefore, subsequent FMA and BPQ data were also removed to ensure, as best as possible, a homogenous data collection protocol. Patients were also excluded from participation if they tested positive to any pathogens known to cause significant health issues, such as *Clostridium difficile*, *Salmonella* species, and *Giardia intestinalis*. Figure 1 presents an overview of the current study's inclusion and exclusion criteria.

Figure 1.

Inclusion and exclusion criteria flowchart.



4.2 Materials and Procedure

4.2.1 Symptom Checklist: Bioscreen Patient Questionnaire (BPQ)

Given that the current thesis is based on a retrospective dataset provided by Bioscreen, the BPQ was the only measure of symptom expression that was used. The BPQ is an 88 item symptom checklist (see Appendix A) which was developed for symptom screening of all patients referred to

Bioscreen regardless of their clinical presentation. Items on the BPQ reflect a wide range of symptoms, similar to the Symptom Checklist 90-Revised (Derogatis, 1992) and the Beck Depression Inventory-II (Beck et al., 1996). Patients were asked to rate both the severity of their symptoms over the last seven days and the frequency of their symptoms over the last 12 months. Both severity and frequency were measured on a 5-point Likert scale ranging from 0 (none at all) to 4 (extremely severe or constant). For the purposes of the current thesis, only symptom severity was explored given the underlying assumption that the temporality of symptom severity over the last seven days is more likely to be related to the patient's microbial composition at that particular point in time, compared to symptom frequency over the previous 12 months. As such, Bioscreen patients were asked to complete the BPQ within temporal proximity of their faecal sample collection.

In a study by Wallis et al. (2016), the BPQ was used to explore the relationship between symptom expression and selected microbial genera in a sample of patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). However, the symptom factors used by Wallis et al. (2016) were clinically classified into 12 factors in accordance with the International Consensus Criteria (Carruthers et al., 2011), plus a mood factor. Because Wallis et al. (2016) were investigating a clinical sample with a known diagnosis of ME/CFS it was appropriate for clinical factors to be derived that were based upon that specific diagnosis. As the specific diagnosis, or even the current health status of participants was unknown for the data used in this thesis, it was important to use mathematically derived factors. Additionally, Wallis et al. (2016) calculated a symptom impact score by multiplying symptom severity over the last seven days and symptom frequency over the last 12 months. Again, while this may have been appropriate given the specific diagnosis of their sample, an impact score was not considered appropriate for the studies presented within the current thesis. This decision was predominantly based on the fact that symptom severity and frequency were measured on two different time scales.

Resultantly, the BPQ has not previously been subjected to statistical analysis in published work. Therefore, the symptom severity items of the BPQ were subjected to an EFA using the maximum likelihood (ML) method of extraction with an oblique (direct oblimin) rotation. The ML method of extraction was chosen given that the aim was to reveal latent variables underlying the data. This differs from principal components analysis which is primarily a data reduction method which does not discriminate between shared and unique variance (Costello & Osbourne, 2005). The results of the EFA are presented in online resource 1 for Paper 2 (Ganci et al., 2021). In short, ten symptom domains were identified (four psychological and six physiological). For the purposes of the current thesis which intended to investigate the relationship between GM and psychological

symptom expression, only the four psychological symptom factors were used within the studies presented within. The psychological symptom domains derived were Depressive, Neurocognitive, Sleep and Fatigue, and Stress and Anxiety. The number of items, possible range of scores, and associated Cronbach's alpha can be found in Table 2.

Table 2.

Psychological symptom factors derived from EFA of the BPQ.

Symptom domain	Items	Possible range	Cronbach's alpha
Depressive	6	0 – 24	.89
Neurocognitive	8	0 – 32	.94
Stress and Anxiety	9	0 – 36	.87
Sleep and Fatigue	6	0 - 24	.85

As can be seen in Table 2, all four psychological symptom factors demonstrated Cronbach's alpha values well above the minimum acceptable values of 0.6 to 0.7 (Taber, 2018). For each of the factors, lower scores represent lower symptom severity in that domain, while conversely, higher scores indicate greater severity of symptoms.

In unpublished work undertaken by the broader research team, an EFA was conducted on the BPQ to determine its convergent validity with established measures. The factors derived were very similar to those derived in the current thesis. The cognitive factor (in this thesis labelled as Neurocognitive) was validated against the Cognitive Failures Questionnaire (CFQ). The sleep factor (in this thesis labelled as Sleep and Fatigue) was validated against the Pittsburgh Sleep Quality Index (PSQI). Finally, the depression, anxiety, and stress factors (in this thesis labelled as Depressive, and Stress and Anxiety) were validated against the subscales of the Depression, Anxiety, and Stress Scales (DASS-21). Each factor demonstrated positive moderate-to-strong significant relationships with the relevant comparable measures, demonstrating good convergent validity (Abma et al., 2016), see Appendix B.

4.2.2 Sample Collection and Analysis

Stool samples were collected by patients according to directions provided in a Bioscreen FMA kit which was posted to their home. Specific details regarding collection, storage, and transportation are discussed in Paper 2 and Paper 3.

Faecal Microbial Analysis (FMA). The FMA process described here was identical to that used in Coulson et al. (2013) and Wallis et al. (2016). Given that the current study extends on work previously conducted within the same research team at Victoria University by Wallis et al. (2016), the procedures outlined below are exactly the same. The FMA process is detailed in the method and procedure section of Paper 3. Given the specificity of FMA, the following information has been sourced from Coulson et al. (2013) and Wallis et al. (2016).

Briefly, and to provide context, FMA is a culture-based method of analysing GM. Essentially, stool samples are processed, diluted, and transferred onto culture plates with a variety of different mediums to foster growth. Samples were then analysed using a Microflex matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (Bruker Daltonik GmbG, Leipzig, Germany). Only the most prevalent microorganisms were quantified as colony forming units per gram of stool (CFU/g). A CFU is a unit used in microbiology to estimate the viable (live) bacterial or fungal cell counts in a sample. The lowest detectable limits provided by Bioscreen as they relate to different microorganisms can be found in Appendix C. In the instance that an individual's CFU count was lower than the minimum detectable limit for a particular species, this would result in a missing value for that species. This does not necessarily mean that the microorganism was not present, but that it was not present in sufficient numbers to be detected.

Protozoa - Polymerase Chain Reaction (PCR) Test. In testing for the presence of intestinal protozoa, a different method was utilised. The PCR method differs from FMA in that it detects genetic material (DNA). The PCR process is outlined in the method and procedure section of Paper 2.

4.3 Data Management

4.3.1 Data Screening and Cleaning

Extensive data screening and cleaning was undertaken prior to any analyses being completed using the dataset. The dataset used in the studies within the current thesis was retrospectively collected over 2 and a half years (January 2013 and June 2015), and put together by a number of contributors (including employees of Bioscreen and research assistants). As might be expected with such a large dataset, there were inconsistencies in data entry which needed to be rectified. For example, in some instances where a bacterial species was not found at detectable limits, an arbitrary value of "8" was prescribed in the dataset. However, this was not applied consistently. As such, any values of "8" were removed from the dataset as these values were distinctly different to the CFU/g counts of detected bacterial species which were generally $1x10^5$ or greater. Any values which had been originally entered as "8" were treated as missing.

4.3.2 Dealing with Missing Data

Missing data in studies of the microbiota is inevitable, however in no paper reviewed for this thesis have any authors addressed the amount of missingness within their data, nor explained how they handled it. Of the more than 2000 microbial species that have been identified in the gut (Almeida et al., 2019), it has been proposed that an individual's GM consists of 100 to 500 different species (e.g., King et al., 2019; Quigley, 2013). As such, two individuals are extremely unlikely to have the exact same composition of microorganisms. As samples increase in size, the heterogeneity of GM composition also increases.

The problem of missing data was a particularly vexing issue for this thesis. The amount of missing data for microbial counts in the dataset varied from 32% to 99%. This issue may be exacerbated by the fact that MALDI-TOF databases are updated when new species are identified. This means that FMA of patients in 2015 may have been conducted with an updated database which was able to detect more species than the FMA of patients in 2013. However, this is only one possibility. Consultation was made with the microbiologists at Bioscreen who explained that absence of a particular organism for any patient sample does not necessarily mean that the microorganism was not present, rather that it was not present at detectable limits. They explained that any microorganism not included in the patient result could in fact be completely absent, or present in the millions, but still at a level just below detectable limits. This made the question of how to handle missing data particularly important to the integrity of the dataset, and the analyses that could be undertaken. Several options were explored, and these are explained below.

Multiple imputation was the first method considered towards rectifying the problem of missing data. Madley-Dowd et al. (2019) argue that the proportion of missing data should not guide decisions in the use of multiple imputation, and suggest that such strategies can be used even with up to 90% missing data. However, this only applies to cases where data are missing at random, and there are sufficient auxiliary variables. Auxiliary variables refer to any variable included in the original dataset that are not being analysed, but are related to the variables in the analysis (Hardt et al., 2012). Given the retrospective nature of the data used in the current thesis, it was not possible to collect additional data which could have served as auxiliary variables. The data was analysed to determine the type of missingness, specifically, whether the data were missing completely at random (MCAR). To do so, Little's MCAR test was used which demonstrated a statistically significant value ($p < .001$). A statistically significant value indicates that data were not missing completely at random (Coertjens et al., 2017). This suggests that the missingness is in some way systematic (missing values are related to the value for a given variable). Additionally, Kaul et al. (2017) suggest that missingness in microbiological variables cannot be treated according to typical

conceptualisations of missingness. This is due to the missingness being a result of a person's underlying biology, rather than being related to the variable itself. As such, traditional imputation methods would be erroneous (Kaul et al., 2017). This would be further exacerbated by the amount of missingness present in the current dataset. Given the amount of missing data, the fact that data were not found to be MCAR according to typical notions of missing data, and a lack of auxiliary variables, multiple imputation was therefore discarded as a viable option.

In consideration of all the aforementioned points, the decision was made not to replace the missing data, and to only use data that were available. Jakobsen et al. (2017) suggest that when there are large proportions of missing data (40% or more), complete case analysis is a more appropriate alternative. While complete case analysis typically refers to listwise deletion, this technique would have resulted in zero available cases for analysis, as no two patients had information for an identical set of microorganisms. Therefore, pairwise analysis was selected to allow for investigation of the relationships between viable CFU/g counts of individual microorganisms and psychological symptom severity where possible.

Another strategy explored attempted to deal with the missing data by replacing any missing values with a fixed value based on minimum detectable limits for the specific bacteria. In consultation with Bioscreen, a prescribed value of one exponent of power less than the minimum detectable limit was intended to replace missing data. For example, the lowest detectable limit for *Bifidobacterium* was $5x10^8$, therefore, for any species of *Bifidobactrium* that a participant was missing, a value of $5x10^7$ was proposed to be added. However, again due to the proportion of missingness, this heavily skewed the data and substantially reduced the variance as the result was to have up to 99% of many variables showing the identical value. This also resulted in manufactured relationships. For example, those where a relationship between a microbial species and any of the psychological symptom domains was found where only a single patient in the dataset showed a detectable CFU/g count of that species. As such, any significant result could only be due to the imputed values. Additionally, this approach had not previously been used in the literature, and the replacement values were deemed to be too arbitrary to be meaningful, and so this method was also discarded.

As such, the results of Paper 2 and 3 are intended to be interpreted as exploratory and associative only.

4.3.3 Choice of Data Analysis Methods

Extensive efforts were made to use advanced statistical methods such as cluster analysis, latent class analysis (LCA) and structural equation modelling (SEM) to interrogate the data in an in-

depth manner. The original contention of this thesis was to investigate clusters of GM that were associated with psychological symptom factors. Unfortunately, the amount of missing data with regards to the microorganisms, and the nature of the missingness precluded the ability to do so. In order to conduct SEM, a complete dataset with no missing data is a core assumption. Typically, various missing data techniques could be applied to complete a dataset, however, as suggested by Kaul et al. (2017), traditional imputation methods would be erroneous. This would be further exacerbated by the amount of missingness present. The following sections outline the limitations relevant to each statistical technique considered.

The Pursuit of GM Phenotypes. Originally, the intention was to use cluster analysis to group participants based on characteristics of their GM at the species level. Given the number of species identified, it was not possible to cluster all GM species. This was further complicated by the amount of missingness, which meant that even with an overall sample of 4610, when submitting the data to a cluster analysis, a warning message was provided informing that there were not enough valid cases to conduct the specified analysis. As such, it would only have been possible to conduct a cluster analysis with a handful of microorganisms that would have been arbitrarily selected. Consideration was given to clustering the top 10 most abundant or frequently occurring species within the dataset, however this was deemed to be too arbitrary to produce meaningful findings, as even low abundance microbes can impact on the structure and functions of the overall microbial community (e.g., de Cena et al., 2021). Furthermore, attempts to triangulate the decision to select specific microbes to cluster with literature regarding common GM was inconsistent, therefore cluster analysis was not considered to be appropriate to address the research aims.

LCA is similar to cluster analysis in that it aims to categorise individuals into distinct classes by finding heterogeneity within a population. However, rather than the hierarchical algorithms used in cluster analysis, LCA is based on probabilistic modelling (Petersen et al., 2019). It therefore provides further information regarding the fit statistics of the classes/groups, making them more accurate than clusters derived in cluster analysis. However, when attempting to conduct LCA on the data, similar problems were faced. That is, the variables within the dataset were far too numerous, and the amount of missingness meant that the analysis was unable to be performed. This, again, would have meant that a smaller number of arbitrarily chosen microorganisms would have had to be chosen for analysis, which was not considered to be capable of producing meaningful results.

An inverse approach was to investigate the possibility that psychological symptom expression may have been clustered to form meaningful groups that may associate with GM frequency and composition. Notwithstanding the same implications of missingness inherent in the

GM data, psychological symptom expression was considered an inappropriate and less meaningful independent variable in relation to the research aims.

Structural Equation Modelling (SEM). SEM is a framework which combines several multivariate statistical procedures including regression analysis, path analysis, and factor analysis (Stein et al., 2017). This makes SEM a comprehensive statistical approach to examining complex relationships among variables (Hoyle, 1995). Datasets with a large number of variables and small sample sizes pose several problems in the use of SEM (Deng et al., 2018). While there were 4610 participants overall, not a single microbial variable was detected in all participants. Moreover, when considering varying combinations of even just a few microbes, the number of participants who had those specific combinations was drastically smaller. The original intention was to first use cluster analysis or LCA to reduce the number of microbial variables. As this was not feasible, this posed a problem for the subsequent use of SEM. However, perhaps the most significant issue preventing the use of SEM in the current thesis was the missing data. In order to conduct SEM, a complete dataset is required. Several methods for dealing with missing data have been proposed (e.g., Jia & Wu, 2019), however none of these were appropriate for the current data (Kaul et al., 2017).

4.3.4 Analyses Used in the Current Thesis

Multivariate Analysis of Variance (MANOVA). In Paper 2, the objective was to compare the self-reported psychological symptom severity of patients who had tested positive for intestinal protozoa Blastocystis, D. fragilis, or both to those who had tested negative. Unlike information regarding FMA results which provided continuous CFU/g counts, data provided for intestinal protozoa was categorical (present/not present) lending itself to use as an independent variable. Given that there were four psychological symptom domains measured, a MANOVA was selected as the most appropriate option. More specifically, as the aim of Paper 2 was to determine whether the effect of protozoan carriage differed as a function of sex, a two-way MANOVA was employed.

Correlational Analysis. While there was substantial missingness, there was also a lot of valuable data that was present. Therefore, the focus shifted to determining how the data that was present could be meaningfully analysed. It was determined that correlational analyses using pairwise deletion would make the best, and most meaningful, use of the available data. While pairwise deletion is not ideal as it may increase parameter bias and can reduce statistical power (which are also the consequence of missing data itself; Kang, 2013), it was the only viable option in dealing with the missing data. While even missing data techniques labelled as “state of the art” are still imperfect (Newman & Cottrell, 2015), it’s important to reiterate that the findings within paper 3 are intended

to be interpreted as exploratory. The choice of correlational analysis is explained in the data handling and statistical design section of Paper 3.

4.3.5 Assumption testing

The dependent variables (Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue symptom domains) were tested for normality. Kim (2013) suggests that for large sample sizes (>300) formal normality tests such as Shapiro-Wilk and Kolmogorov-Smirnov may be unreliable, while interpretation of skewness and kurtosis is more appropriate. Kim (2013) suggests a skewness value of 2 and a kurtosis value of 7 as reference points to indicate non-normality. Other more conservative estimates suggest that acceptable skewness and kurtosis limits of ± 2 (Gravetter & Wallnau, 2014). The skewness and kurtosis values for all four dependent variables (symptom domains) were well within these limits (> -1 and < 1). Specific assumptions relating to the use of MANOVA are mentioned in Paper 2.

In exploring microbial counts (as opposed to the dichotomous presence or absence of intestinal protozoa in Paper 2), Paper 3 needed to contend with violations of normality which are ubiquitous to microbiological data. Typically, log transformations are applied to microbial counts to deal with such violations (Gao & Martos, 2019). Log transformations also help to de-emphasise outliers, which are also common in biological data (Mangiola et al., 2021; Metcalf & Casey, 2016). It is not surprising that outliers are common, particularly in microbiological data, given the vast spread of interindividual differences in GM composition, and the number of factors that exert an influence over said composition. However, log transformations were not required for the correlational analysis utilised in Paper 3, as Kendall's Tau-b (τ_b) was used, which is a non-parametric analysis that is robust to both violations of normality and outliers (van Doorn et al., 2018).

Chapter 5: The effect of *Blastocystis* sp. and *Dientamoeba fragilis* on psychological symptom severity in a sample of clinically diverse males and females

Paper 2 addresses the first research question which relates to determining whether intestinal protozoa, specifically *Blastocystis*, *D. fragilis*, or co-carriage of the two, would impact on symptom severity.

This study provides an important first step into better understanding the relationship between GM and symptom severity. The majority of BGMA research to date focuses exclusively on the bacterial component of the gut ecosystem. However, given that the GM is a complex ecosystem which is not just comprised of bacteria, attention must be given to the other members of this community, such as protozoa. Investigating these non-bacterial members of this ecosystem will improve the overall understanding of host-microbiota relationship. While addressing this important gap in the literature, this paper also informs the inclusion/exclusion criteria for Paper 3.

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The effect of *Blastocystis* sp. and *Dientamoeba fragilis* on psychological symptom severity in a sample of clinically diverse males and females

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Abstract

Health outcomes associated with *Blastocystis* sp. and *Dientamoeba fragilis* are disparate and controversial, ranging from health benefits, to years of asymptomatic carriage, through to severe illness. Evidence that *Blastocystis* sp. and *D. fragilis* are commensal members of the gut microbiota is growing. Despite this, little to no research exists investigating the potential effect of these protozoa on psychological symptom expression. As such, the aim of this retrospective cross-sectional study was to be the first to investigate the effect of protozoan carriage on severity of Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue symptoms, and whether this effect changes as a function of sex. The prevalence of *D. fragilis* was significantly higher in females compared to males, however there were no sex differences in prevalence for *Blastocystis* sp. (data used in the current study contained ST1, ST3, and *Blastocystis* ST unspecified) or co-carriage of the two. Females reported significantly more severe symptoms across all four psychological domains compared to males. There was no significant interaction between sex and *Blastocystis* sp. carriage on psychological symptom severity, and no significant main effect of *Blastocystis* sp. on symptom severity compared to those who tested negative for protozoa. When investigating the sexes separately, there was no effect of protozoan carriage on psychological symptom expression in either males or females. These findings add weight to the argument that *Blastocystis* sp. and *D. fragilis* are not necessarily pathogenic and are likely to be part of a diverse gut (which is typically associated with better health outcomes). Further research is required given that protozoan members of the gut microbiota have been largely ignored in brain-gut-microbiota axis research.

Keywords *Blastocystis* · *Dientamoeba fragilis* · Gut-brain axis · Psychological symptoms

Introduction

Research into the brain-gut-microbiota axis (BGMA) has uncovered multidirectional relationships between the microorganisms residing in the gut and the development and functioning of the central nervous system (e.g. El Aidy et al., 2016; Rea et al., 2016). Alterations in gut microbiota have been associated with mood (Jiang et al., 2015) and developmental disorders (Li et al., 2017) as well as neurodegenerative (Vogt et al., 2017) and neuroimmune conditions (Giloteaux et al., 2016). However, the vast majority of BGMA research has focused solely on the bacterial component of this ecosystem. While research has been undertaken investigating associations between eukaryotic protozoa and gastrointestinal (GI) disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) as well as other GI symptoms, there is limited research into the influence of protozoa on psychological functioning. Further still, there does not appear to be any research to date which has focused specifically on *Blastocystis* sp. and *Dientamoeba fragilis*, two commonly detected intestinal protozoa (e.g. Aykur et al., 2019; Tito et al., 2019).

A growing body of evidence suggests that protozoa *Blastocystis* sp. and *D. fragilis* are commensal members of the gut microbiota that have coevolved with their human host (e.g. Beghini et al., 2017; Chabé et al., 2017; Scanlan et al., 2014). A number of studies have demonstrated either no differences in the prevalence of *Blastocystis* sp. and/or *D. fragilis* in asymptomatic groups compared to those with IBS, IBD, or other GI symptoms

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(e.g. Jokelainen et al., 2017; Seyer et al., 2017) or even a higher prevalence of these protozoa in asymptomatic groups (e.g. Beiromvand et al., 2017; Brands et al., 2019; Holtman et al., 2017; Rossen et al., 2015; Tito et al., 2019). One possible explanation for the protective nature of these protozoa is the association between carriage of *Blastocystis* sp. and/or *D. fragilis* and higher gut bacterial richness and diversity (Audebert et al., 2016; Krogsgaard et al., 2018; Tito et al., 2019) which are indicators of a healthy microbiota (e.g. Bruce-Keller et al., 2018; Lloyd-Price et al., 2016; Rinninella et al., 2019; Valdes et al., 2018).

From an evolutionary perspective, intestinal microorganisms that have coevolved with their human host, including protozoa, may have immunomodulating effects (Chudnovskiy et al., 2016; Deng et al., 2021; Maizels, 2009). The 'old friends' hypothesis (Rook & Brunet, 2005) proposes that lower exposure to immunoregulating intestinal microorganisms may be related to the higher prevalence of chronic and inflammatory diseases seen today (Chabé et al., 2017; Rook, 2013; 2014). This is supported by the higher incidence of inflammatory and allergic diseases and lower prevalence of intestinal protozoa seen in industrialised countries compared to non-industrialised countries that maintain a more traditional lifestyle, which have a higher prevalence of protozoa and lower incidence of inflammatory and allergic disease (Chabé et al., 2017; Lokmer et al., 2019). However, coevolution can alternatively lead to an evolutionary arms race; a cycle of adaptations and counter-adaptations between host and protozoan (Dawkins & Krebs, 1979; Laanto et al., 2017). In contrast to the 'old friends' hypothesis which proposes immunological benefits for the human host, immune responses induced by *Blastocystis* sp. may instead result in increased symptomatology (El-Zawawy et al., 2020). Currently, knowledge regarding the immune response to *D. fragilis* remains lacking (Yadav et al., 2020), contributing to its label as a neglected protozoan (e.g. Al-Hindi & Abu-Shammala, 2013; Garcia, 2016) and to the uncertainty over its pathogenicity.

As such, controversy remains over the potential pathogenicity of these protozoa (e.g. Garcia, 2016; Lepczyńska et al., 2017) with other studies finding an increased prevalence in groups with GI symptoms (Crotti & D'Annibale, 2007; Kesuma et al., 2019; Norberg et al., 2003). These inconsistencies may be due to several factors which could alter the pathogenicity of these otherwise commensal organisms including the bacterial component of the gut microbiota (Burgess et al., 2017), protozoan load (Pavanelli et al., 2015), duration of carriage (Kaneda et al., 2000; Lukeš et al., 2015), and the sex and immune functioning of the host (Chandramathi et al., 2012; Nourrisson et al., 2014). Specific protozoan subtypes (STs) have also been associated with varying symptom outcomes (Bart et al., 2013; Zulfa et al., 2017). *Blastocystis* sp. is one such example with 17 subtypes (STs) identified, of which STs 1–9 and ST12 have been identified in humans (Jiménez et al., 2019; Stensvold & Clark, 2016; Tito et al., 2019). Chandramathi et al. (2014) also demonstrated that stress can exacerbate the pathogenic potential of *Blastocystis* sp.

Despite the continued debate about the pathogenicity of *Blastocystis* sp. and *D. fragilis*, typical treatments for these protozoa include administering antimicrobial agents (such as metronidazole and trimethoprim-sulfamethoxazole among several others; Coyle et al., 2011; Nagata et al., 2012). However, these treatments have potentially serious side effects such as confusion and headaches, nausea, vomiting, and may disrupt the delicately balanced microbial ecosystem in the gut (Ho & Juurlink, 2011; Weir & Le, 2020). Treatment for asymptomatic individuals is therefore not recommended (Coyle et al., 2011).

Comparatively, psychological outcomes of *Blastocystis* sp. and/or *D. fragilis* carriage have received little to no attention, particularly in humans. There is only very limited evidence of a possible link between these protozoa and fatigue (Johnson et al., 2004; Norberg et al., 2003; Qadri et al., 1989). However, this evidence is weak as it typically comes from case reports (Butler, 1996; Dunwell, 2013) or studies where other possible causes, such as IBS, were not ruled out (Norberg et al., 2003). Conversely, Holtman et al. (2017) found an association between the absence of *D. fragilis* and fatigue in children, however they offered no viable explanation for this result.

In regard to psychological symptom expression, research suggests that there are differences between males and females, with females tending to report more sleep problems (Boccabella & Malouf, 2017) and have a greater risk of anxiety and depression (Altemus et al., 2014). It is suggested that sex hormones interact with stress hormones in a way that makes females more vulnerable to stress-related disorders (Solomon & Herman, 2009). Additionally, Hall and Steiner (2013) explain females' greater susceptibility to various psychopathologies by suggesting that sex hormones, particularly fluctuations in estrogens, may interact with the serotonergic system. Sex differences in immunoregulatory function (Sorge & Totsch, 2017) and symptom expression and reporting (Barsky et al., 2001; MacLean et al., 2010) must also be considered in relation to the effect of protozoan carriage on symptom expression (e.g. Maeng & Milad, 2015; Mulak et al., 2014; Seney & Sibille, 2014; Sorge & Strath, 2018). This is, due to the immunoenhancing effect of estrogen, and the immunosuppressive effects of testosterone (Kovats, 2015; Taneja, 2018), which may influence susceptibility to, and the consequential effects of, intestinal protozoa. In addition to sex differences in symptom expression, the microgenderome is a paradigm highlighting sex specific host-microbiota interactions (Flak et al., 2013; Markle et al., 2013). Research focusing on the bacterial component of the microbiota has provided support for this paradigm (e.g. Vemuri et al., 2019; Wallis et al., 2016). Studies investigating protozoan carriage have provided further support for the microgenderome with different health outcomes reported between males and females (Klein, 2004; Marriott & Huet-Hudson, 2006; Salehi et al., 2021; Zuk, 2009). Nourrisson et al. (2014) found that *Blastocystis* sp. was associated with

IBS only in males, and that carriage was also associated with a reduction of *Bifidobacteria* and *Faecalibacterium prausnitzii* in male control group participants. Generally, protozoan carriage is more prevalent in males (Abu-Madi et al., 2016; Klein, 2004), however there are some exceptions, such as *D. fragilis,* which trends towards higher prevalence in females (Barratt et al., 2011; Crotti & D'Annibale, 2007; Grendon et al., 1995; Röser et al., 2013). However, there are inconsistencies within the literature with some studies showing relatively equal prevalence of *D. fragilis* in males and females (Al-Hindi & Abu-Shammala, 2013; Hamidi et al., 2018; Stark et al., 2010). As such, sex differences in susceptibility to, carriage of, and health outcomes associated with protozoa remain poorly understood and more research is needed.

While the majority of studies investigating intestinal protozoa *Blastocystis* sp. and *D. fragilis* have thus far centred their investigation on GI symptoms, this study served to take an essential first step to fill a gap in the literature investigating the influence of these two protozoa on psychological symptom severity across Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue symptom domains. The aim of the current study was to determine whether there is an effect of *Blastocystis* sp. and/or *D. fragilis* carriage on self-reported psychological symptom severity, and whether this effect changes as a function of sex. Given that this is the first study to the authors' knowledge which has investigated the influence of these specific protozoa on psychological symptom outcomes, this study is exploratory, and as such, what is to be expected is somewhat uncertain. However, given the increasing evidence that *Blastocystis* sp. and *D. fragilis* are part of a healthy gut (e.g. Chabé et al., 2017) the following hypotheses are put forward. Firstly, it was hypothesised that carriage of *Blastocystis* sp. and/or *D. fragilis* would not have a detrimental effect on symptom severity. Secondly, it was hypothesised that females would self-report more severe symptoms compared to males in all four psychological domains. Finally, in line with research in support of the microgenderome, it was hypothesised that the effect of protozoan carriage on symptom expression would differ as a function of sex.

Materials and Methods

Study Participants

Data analysed in the current study was taken from a larger retrospective database of 4610 clinically diverse adult (18 years or above) patients who were referred to Bioscreen, a Melbourne based laboratory specialising in faecal microbial analysis (FMA), between February 2013 and June 2015. The subsection of the sample used in the current study were those whose stool samples were tested for intestinal protozoa (n = 988). Stool samples were submitted for analysis as part of the investigatory process for intestinal dysbiosis however, the specific diagnostic status of patients were not identified. As such, this is a cross-sectional study of a broad range of potentially clinical and non-clinical presentations. Ethics approval for the current study was obtained from the Victoria University Human Research Ethics Committee (HRE16–071).

Inclusion and Exclusion Criteria

From the larger database, inclusion criteria for the current study was that two-step polymerase chain reaction (PCR) testing was conducted to indicate the presence or absence of intestinal protozoa, that patients completed the Bioscreen patient questionnaire (BPQ), and that patients also provided consent for their information to be used in future research. Patients were excluded from the current study if PCR testing, BPQ data, or consent were not provided, or if their stool sample was unsuitable for testing as defined by quality control guidelines. A further exclusion criterion was if patients tested positive for intestinal protozoa other than *Blastocystis* sp. or *D. fragilis* (including *Cryptosporidium* and *Giardia*; n = 9) or any other known pathogens (e.g. *Clostridium difficile*). No further information was available to exclude other possible extraneous of confounding variables.

Overall, 979 patients met the criteria for inclusion in the current study. Available demographic information for the sample is presented in Table 1.

Sample Collection and Parasite PCR Assay

Stool samples were collected by patients according to directions provided in a Bioscreen FMA kit which was posted to their home. Patients stool samples were kept anaerobically and transported via express post (typically next day delivery) to the laboratory in cool conditions (below 12 °C). Patients were instructed to complete the BPQ within temporal proximity to faecal sample collection and return this with the kit. On receipt, samples were kept at −20 °C until tested (within three days). Samples were subjected to internal quality assurance and were rejected if they had been inaccurately collected, transported, refrigerated, or delayed in transit.

Table 1 Sample demographic information

Sex	n (%)	Age range	Mean age
Male	258 (26.4)	18–82	43.582 (SD=14.013)
Female	721 (73.6)	18–86	42.796 (SD=13.124)
Total	979	18–86	43.003 (SD=13.361)

On the day of testing, faecal samples were thawed at room temperature. A custom-made 7-well target panel was used to screen for *Blastocystis* sp., *Blastocystis* subtype (ST) 1 and 3, *D. fragilis, Entamoeba histolytica, Cryptosporidium,* and *Giardia* (Ausdiagnostics Pty Ltd., Sydney, Australia). DNA extractions were carried out using a QIAamp DNA Stool Mini Kit and QIAcube (Qiagen, Melbourne, Australia). In short, 200 mg thawed samples were homogenised using inhibitEX buffer (Qiagen) and centrifuged. A Two Step Multiplex PCR was carried out as per the manufacturer's instructions (Ausdiagnostics). Amplification using primers homologous to all targets in the panel were used. Gene targets for each assay can be found in Table 2. Real-time PCR was automated by the Easy-Plex system (AusDiagnostics). The Ultra-Plex analyser (Ausdiagnostics) was used for DNA amplification.

Faecal Microbial Analysis (FMA)

The FMA process used in the current study was identical to that performed in previous studies conducted by Coulson et al. (2013) and Wallis et al. (2016). In brief, culture methods, on a variety of media (previously dried Columbia horse blood agar (Oxid, Thermo Fisher, Australia), chromogenic medium (Oxid), colistin and nalidixic acid blood selective agar (Oxid), and chloramphenicol-gentamicin selective Sabouraud agar), were used to perform bacterial counts within 48 h of sample collection. Specifically, Matrix Assisted Laser Absorption and Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS) using a proprietary peptide database (MALDI Biotyper Bruker Daltonics, Bremen, Germany) was utilised for identification.

Bioscreen Patient Questionnaire (BPQ)

The BPQ is an 88-item questionnaire developed by Bioscreen administered to patients as part of Bioscreen's standard procedure. Items on the BPQ are similar in nature to other symptom checklists which relate to diverse symptomatology and patients are asked to report the frequency (over the past 12 months) and severity (over the past seven days) of their symptoms on a five-point Likert scale ranging from zero to four, with higher scores indicating higher ratings of frequency/severity. For the purposes of the current study, only symptom severity over the past seven days was assessed, given the underlying assumption of a temporal relationship between protozoan carriage and symptom expression. This is the first study to use mathematically derived symptom factors from the BPQ. The methods used as well as sub-scale reliabilities are outlined in the data handling and statistical design section.

Table 2 Assay gene targets

Assay	Gene target
E. histolytica	Surface antigen ariel 1
D. fragilis	18S
Cryptosporidium	Oocyte wall protein
Giardia	18S
Blastocystis ST 1	Small subunit ribosomal RNA
Blastocystis ST 3	Small subunit ribosomal RNA
BlastE1-a	Elongation factor 1-alpha

Data Handling and Statistical Design

Deriving Symptom Domains from the BPQ

Symptom domains were derived mathematically by subjecting the BPQ to factor analysis using the maximum likelihood (ML) method of extraction in the larger sample from which the current sample was drawn (see Online Resource 1). The factor analysis revealed that of the 88 original items, 53 items loaded onto 10 distinct factors, with minimal cross loadings. These 10 factors covered Depressive (6 items; Cronbach's $\alpha = 0.894$), Pain (6 items; Cronbach's $\alpha = 0.849$), Neurocognitive (8 items; Cronbach's $\alpha = 0.938$), GI (5 items; Cronbach's $\alpha = 0.782$), Stress and Anxiety (9 items; Cronbach's $\alpha = 0.874$), Sleep and Fatigue (6 items; Cronbach's $\alpha = 0.853$), Exertion (4 items; Cronbach's $\alpha = 0.820$), Urination (3 items; Cronbach's $\alpha = 0.684$), Neck and Shoulder pain (2 items; Cronbach's $\alpha = 0.847$), and Immunity (4 items; Cronbach's $\alpha = 0.652$) symptom domains. The specific items that loaded onto each symptom domain factor can be found in Table 3 in Online Resource 1. For the purposes of the current study, only the psychological domains (Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue symptoms) were used in all further analyses.

Combining Blastocystis Subtypes (STs)

Prior to any further analysis, a decision was made to combine patients who tested positive for *Blastocystis* ST unspecified, *Blastocystis* ST1, *Blastocystis* ST3, and co-carriage of *Blastocystis* ST1 and ST3 into a single variable, allowing for the analysis of *Blastocystis* sp. at the genus level. While it would be ideal to compare the effect of the various *Blastocystis* STs, this is not possible given the current retrospective database for three main reasons. The first is that it was only specified whether a patient had *Blastocystis* ST1, *Blastocystis* ST3 (or co-carried both ST1 and ST3), or *Blastocystis* ST unspecified with no information being available regarding the other eight *Blastocystis* STs that have been identified in humans. Secondly, for those who tested positive for *Blastocystis* ST unspecified, it cannot be concluded with any degree of certainty which of the 10 *Blastocystis* ST they potentially carried. Finally, the percentage of patients who

Table 3 *Blasocystis* and *D. fragilis* prevalence ($N = 979$)

	Males ($n=258$)		Females ($n=721$)		Total ($n=979$)	
	n	Mean age in years (*SD*)	*n*	Mean age in years (*SD*)	*n*	Mean age in years (*SD*)
Protozoan negative	161	43.90 (14.37)	402	43.20 (13.73)	563	43.40 (13.90)
Blastocystis sp.	75	43.25 (13.39)	199	43.06 (13.16)	274	43.11 (13.20)
D. fragilis	10	44.46 (13.14)	59	38.99 (9.97)	69	39.78 (10.55)
Co-carriage	12	40.62 (14.97)	61	42.95 (11.15)	73	42.57 (11.77)
Total	258	43.58 (14.01)	721	42.80 (13.12)	979	43.00 (13.36)

tested positive for any *Blastocystis* ST ($n = 274$) was 67.2% for *Blastocystis* ST unspecified ($n = 184$), 13.1% for ST1 ($n = 36$), 16.8% for ST3 ($n = 46$), and 2.9% for co-carriage of ST1 and ST3 ($n = 8$). These discrepancies in sample size are further exacerbated when considering the comparison to patients who tested negative to protozoan carriage ($n = 563$), and again exacerbated when separating for sex. It is believed that this decision would allow for the most meaningful analysis, given the data that was available.

As a safeguard to ensure that this would not introduce undue bias into the results, a 3 × 2 MANOVA was conducted to determine if there was a significant difference in the endorsement of psychological symptom severity between the *Blastocystis* STs (*Blastocystis* ST unspecified, *Blastocystis* ST1, and *Blastocystis* ST3) and whether this effect varied as a function of sex (male or female). The MANOVA demonstrated no significant interaction between *Blastocystis* STs and sex, and also showed no significant main effect for *Blastocystis* STs on psychological symptom severity. These findings support the decision to collapse the *Blastocystis* STs into a single variable to allow for investigation of *Blastocystis* sp. at the genus level in subsequent analyses.

Independent and Dependent Variables

The independent variable (IV), protozoan carriage status, was coded as having four levels (protozoan negative, *Blastocystis* sp. positive, *D. fragilis* positive, or co-carriage of *Blasotcystis* together with *D. fragilis*). Due to the low prevalence of *D. fragilis* and co-carriage in males (as seen in Table 3), these groups were excluded from further statistical analysis. Therefore, when males were included in analyses, only two levels of the IV were considered (protozoan negative or *Blastocystis* sp. positive). Factor scores for each of the four psychological symptom domains were calculated and used as the dependant variables (DVs) in three multivariate analyses (MANOVA). The first, was a 2 × 2 (*Blastocystis* sp. [positive or negative] x sex [male or female]) independent measures MANOVA to determine the effect of *Blastocystis* sp. carriage on psychological symptom severity, and whether this effect changed as a function of sex. When separating for sex, differences in psychological symptomatology between males who tested negative for protozoa and those who tested positive for *Blastocystis* sp. were analysed using a one-way independent measures MANOVA. For females, who had a sufficient sample size across all four levels of the IV, a separate one-way independent measures MANOVA was used to compare psychological symptomatology.

Chi-square test of independence was used determine whether there was an association between sex and protozoan carriage, with Cramer's V used to determine the effect size of this association (Akoglu, 2018; McHugh, 2013).

Statistical Assumptions

The assumptions of normality (skewness and kurtosis) and multivariate normality (using the Mahalnobis distance critical value appropriate for four DVs) were assessed and met. The assumption of homogeneity of variance was not violated for all following analyses. Moreover, the Box's M Test of Equality of Covariance Matrices demonstrated that the assumption of homogeneity of variance-covariance matrices was also met for all analyses according to Hubery and Petoskey's (2000) guideline of $p < .005$. Pearson's correlation coefficients in the moderate range demonstrated that multicollinearity did not exist between the four DVs. A linear relationship existed between each pair of DVs. No extreme outliers were detected. Finally, listwise deletion was used to deal with missing data. All analyses were interpreted using an alpha (α) of .05.

Results

Protozoan Prevalence

Information regarding the prevalence of *Blastocystis* sp. and *D. fragilis* is shown in Table 3.

Based on the protozoan distribution among males and females seen in Table 3, chi-square test for independence

showed that there was a significant association between protozoan carriage and sex, $\chi^2(3, N = 979) = 10.305$, $p = .016$. The associated Cramer's V of .103 suggests that the effect size of this association is small to medium (Akoglu, 2018). Interpretation of the adjusted standardised residuals (Beasley & Schumacker, 1995) demonstrated that the prevalence of *D. fragilis* was significantly higher in females compared to males ($p = .020$). The prevalence of co-carriage was also significantly higher in females ($p = .046$). There was no significant difference in the prevalence of *Blastocystis* sp. between males and females, nor was there a significant difference in the number of males and females who tested negative for either *Blastocystis* sp. or *D. fragilis*.

Effect of *Blastocystis* Sp. on Psychological Symptom Expression as a Function of Sex

Presented in Table 4 are the descriptive statistics relevant to the 2 × 2 MANOVA and the one-way MANOVA analysing symptom severity in males. Mean (*M*) and standard deviation (*SD*) symptom severity scores for males and females who tested negative for protozoan carriage and those who tested positive for *Blastocystis* sp. are presented for all four psychological symptom domains. Mean and standard deviation values for *D. fragilis* and co-carriage which relate only to females can be found in Table 4.

A 2 × 2 MANOVA was run to determine whether there was a significant effect of *Blastocystis* sp. carriage on symptom severity compared to protozoan negative patients and whether this effect differed as a function of sex. The results showed no significant interaction between *Blastocystis* sp. carriage and sex on symptom severity. This demonstrates that the effect of *Blastocystis* sp. on psychological symptom severity is similar in males and females. Given the non-significant interaction, the main effects of sex and *Blastocystis* sp. carriage were considered. The results also demonstrated a non-significant main effect for *Blastocystis* sp. on symptom severity, meaning that irrespective of sex, there was no difference in psychological symptom severity between those who were protozoan negative and those who were *Blastocystis* sp. positive. However, there was a significant main effect of sex on symptom severity, *Wilks'* $\Lambda = .951$, $F(4, 719) = 9.182$, $p < .001$, $\eta_p^2 = .049$, meaning that regardless of protozoan carriage status, there was a difference between males and females on psychological symptom severity. Interpretation at the univariate level revealed that females reported significantly more severe Depressive ($F[1, 722] = 18.266$, $p < .001$, $\eta_p^2 = .025$), Neurocognitive ($F[1, 722] = 8.899$, $p = .003$, $\eta_p^2 = .012$), Stress and Anxiety ($F[1, 722] = 8.978$, $p = .003$, $\eta_p^2 = .012$), and Sleep and Fatigue ($F[1, 722] = 33.671$, $p < .001$, $\eta_p^2 = .045$) symptoms compared to males. It should be noted that these effect sizes are small (partial eta squared values between .0099 and .0587; Cohen, 1969; Richardson, 2011), indicating that only a small amount of variance in psychological symptom severity is accounted for by sex alone.

Effect of Protozoan Carriage within each Sex

Males

A one-way independent measures MANOVA was run to determine whether there was a significant difference between males who tested negative for protozoan carriage, and those who were positive for *Blastocystis* sp. on their self-reported psychological symptom severity. As mentioned in the data

Table 4 Mean symptom severity scores for protozoan negative and *Blastocystis* sp. positive males and females

Symptom domain (possible range)	Protozoan status	Males (M)			Females (F)			Total combined across M and F		
		n	*M*	*SD*	*n*	*M*	*SD*	*n*	*M*	*SD*
Depressive(0–24)	Negative	146	7.137	6.478	346	9.127	6.958	492	8.537	6.873
	Blastocystis sp.	65	6.354	6.333	169	9.414	6.512	234	8.564	6.594
	Total	211	6.896	6.429	515	9.221	6.810			
Neurocognitive (0–32)	Negative	146	9.843	8.895	346	12.009	9.202	492	11.366	9.157
	Blastocystis sp.	65	9.646	8.219	169	12.201	8.881	234	11.492	8.760
	Total	211	9.782	8.673	515	12.072	9.090			
Stress/Anxiety (0–36)	Negative	146	8.096	8.138	346	10.254	8.212	492	9.614	8.241
	Blastocystis sp.	65	7.939	7.925	169	10.107	8.312	234	9.504	8.241
	Total	211	8.047	8.054	515	10.206	8.237			
Sleep/Fatigue (0–24)	Negative	146	7.952	5.715	346	10.104	4.924	492	9.465	5.259
	Blastocystis sp.	65	6.631	5.030	169	9.722	5.040	234	8.863	5.215
	Total	211	7.545	5.535	515	9.979	4.961			

handling and statistical design section above, males who tested positive for *D. fragilis* or co-carriage of *Blastocystis* sp. and *D. fragilis* were excluded from analysis due to small sample size which precluded meaningful comparisons. Refer to Table 4 for descriptive information relating to symptom severity for protozoan negative and *Blastocystis* sp. positive males. The results demonstrated no significant difference between protozoan negative and *Blastocystis* sp. positive males with regard to their psychological symptom severity.

Females

Descriptive information relating to symptom severity in females can be found in Table 5.

A final one-way independent measures MANOVA was run to determine whether there was a significant difference in self-reported psychological symptom severity between females who tested positive for *Blastocystis* sp., *D. fragilis*, or co-carriage compared to those who tested negative for protozoa. Descriptive information relating to symptom severity in females can be found in Table 5. Results demonstrated that protozoan carriage status had no significant effect on psychological symptom severity.

Interaction between Protozoa and Bacterial Composition (Bacteroidetes and Firmicutes)

Additional analyses were conducted to determine whether bacteria (at the phylum level) moderated the relationship between protozoan carriage and psychological symptom severity. The Firmicutes and Bacteroidetes phyla were selected as moderating variables as these two phyla provide a general snapshot of the bacterial component of the gut microbiota. Several moderation analyses were conducted through the PROCESS macro tool for SPSS (Hayes, 2017). First, the protozoan variable (which had four levels) was dummy coded for use in regression analysis (Cohen et al., 2013). The amounts of Firmicutes and Bacteroidetes were first log-transformed and then mean-centred through the PROCESS macro tool (Hayes, 2017). The results revealed that the level of either Firmicutes or Bacteroidetes did not moderate the relationship between protozoan carriage and psychological symptom severity across any of the four psychological symptom domains for males or females (data not shown). As such, the effect of protozoan carriage was not altered by either phyla.

Discussion

This retrospective study contributes to the mounting evidence that carriage of *Blastocystis* sp. and/or *D. fragilis* do not have a negative effect on symptom expression and that these protozoa are likely to be commensal members of the gut microbiota (e.g. Beghini et al., 2017; Jokelainen et al., 2017; Lukeš et al., 2015). These results need to be interpreted in the context of limitations relating to the retrospective study design and methodological challenges which face all studies investigating protozoa and the BGMA more widely which will be discussed herein. Despite these limitations the current study has extended the scope of symptoms investigated with regard to *Blastocystis* sp. and *D. fragilis* carriage beyond GI alone, and is the first to investigate psychological symptoms (Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue) that have previously received no attention. These

Table 5 Mean symptom severity scores relating to protozoan carriage status in females

Symptom domain(possible range)	Protozoan status	*n*	*M*	*SD*
Depressive(0–24)	Negative	346	9.127	6.958
	Blastocystis sp.	169	9.414	6.512
	D. fragilis	53	7.528	6.259
	Co-carriage	56	9.071	6.907
Neurocognitive	Negative	346	12.009	9.202
(0–32)	*Blastocystis* sp.	169	12.201	8.881
	D. fragilis	53	10.981	7.824
	Co-carriage	56	11.321	8.908
Stress/Anxiety	Negative	346	10.254	8.212
(0–36)	*Blastocystis* sp.	169	10.107	8.312
	D. fragilis	53	8.604	6.698
	Co-carriage	56	9.304	8.228
Sleep/Fatigue	Negative	346	10.104	4.924
(0–24)	*Blastocystis* sp.	169	9.722	5.040
	D. fragilis	53	8.396	5.436
	Co-carriage	56	8.375	5.065

findings inform future research into the BGMA and echo previous authors who highlight the need for further research into *Blastocystis* sp. and *D. fragilis*.

The hypothesis that carriage of *Blastocystis* sp. and/or *D. fragilis* would not have a detrimental effect on symptom severity was supported. It was found that for males, there was no difference in self-reported psychological symptom severity between those who tested negative for protozoan carriage and those who tested positive for *Blastocystis* sp. Due to sample size restrictions, the effect of *D. fragilis* and co-carriage of *Blastocystis* sp. and *D. fragilis* was unable to be analysed in males. Females also showed no differences in self-reported psychological symptom severity between those who were protozoan negative, or those who tested positive for *Blastocystis* sp., *D. fragilis*, or co-carriage of the two. While this study is the first to explore the effect of these specific protozoa on psychological symptom severity, the current findings reflect previous research that demonstrates *Blastocystis* sp. and *D. fragilis* not to be harmful in relation to GI symptoms (e.g. Jokelainen et al., 2017; Krogsgaard et al., 2015; Scanlan et al., 2014; Tito et al., 2019). There is a growing consensus that these intestinal protozoa are common members of a healthy gut microbiota (e.g. Audebert et al., 2016; Krogsgaard et al., 2018), which is in line with the 'old friends' hypothesis (Rook & Brunet, 2005) and the co-evolution of these protozoa with their human host (Chabé et al., 2017). Taking an evolutionary perspective, common protozoa that have coevolved with their human host, such as *Blastocystis* sp. and *D. fragilis*, may also contribute to a healthy gut to ensure their own fitness and survival. Tsaousis et al. (2018) suggest that the dysbiotic gut of IBS patients is an unsuitable habitat for the anaerobic *Blastocystis* sp., meaning that for its own survival, it would benefit from supporting a healthy gut. The same may also hold true for the anaerobic *D. fragilis* (Dunwell, 2013), although this has not been specifically tested. As such, it is suggested that the commensalism exhibited in studies focusing on GI symptoms is reflected systemically.

Also as hypothesised, females reported more severe psychological symptoms across all four domains (Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue). This is consistent with a large body of previous literature that suggests females tend to experience and/or report both physiological and psychological symptoms more than males (e.g. Mulak et al., 2014; Seney & Sibille, 2014; Sorge & Strath, 2018). This is believed to be a product of biological sex differences which include interactions between sex hormones (particularly estrogens) both stress hormones (Solomon & Herman, 2009) and the serotonergic system (Hall & Steiner, 2013). Socialised gender expectations are also believed to play a role in greater reporting of various psychological symptoms in females (Barsky et al., 2001). However, the small effect sizes found in the current study suggest that sex itself only explains a small amount of the variance in self-reported psychological symptom severity and that there are likely to be other contributing factors of greater relevance or importance.

Finally, the hypothesis that the effect of protozoan carriage on self-reported symptom expression changes as a function of sex was not supported. However, as the sample of males who tested positive for *D. fragilis* and co-carriage was insufficient for meaningful comparisons to be drawn, the analysis was limited to the interaction between sex and *Blastocystis* sp. The results demonstrated that *Blastocystis* sp. had a similar effect on self-reported psychological symptom expression in males and females and as such does not offer support for the microgenderome. This finding contrasts those of Nourrisson et al. (2014) who found *Blastocystis* sp. to be related to IBS and a reduction of protective anti-inflammatory bacteria only in males. However, this result should not be taken as evidence against the microgenderome more broadly, as these findings relate to *Blastocystis* sp. in exclusion of all other factors (including other intestinal microbiota) and as such results may differ when considering the protozoan in the context of these other factors. With regard to *D. fragilis*, the disparity in prevalence between males and females makes it highly possible that sex hormones may interact with the protozoan, and possibly other intestinal protozoa. Further research is needed to explore these possibilities, as this may have important implications for faecal microbiota transplantation (FMT) donor selection and screening procedures.

In line with previous findings by those such as Crotti and D'Annibale (2007) and Grendon et al. (1995), the main point of difference between the sexes was the higher prevalence of *D. fragilis* in females. The higher prevalence of *D. fragilis* in females is despite the fact that, in general, males are more susceptible to protozoan and parasitic infections as a result of immunosuppressive androgens, while immunoenhancing oestrogen is proposed to contribute to resistance in females (Klein, 2004). However, the argument of immune resistance may not apply to *Blastocystis* sp. and *D. fragilis* which further supports their role as commensal organisms. If considering *Blastocystis* sp. and *D. fragilis* to be commensal members of the gut microbiota that have co-evolved with their human host, the immune system should not respond in the same way as it would to pathogenic protozoa and parasitic infections. This would explain the similar prevalence of *Blastocystis* sp. found in males and females, but still does not explain the increased prevalence of *D. fragilis* in females. Instead, this may be explained by cultural and behavioural differences between males and females (Barratt et al., 2011). For example, females tend to have close and frequent contact with young children (Rhoads & Rhoads, 2012) which may make them more susceptible. Supporting this argument, Röser et al. (2013) found that only females of parental age had a significantly higher prevalence of *D. fragilis*, while Menendez Fernandez-Miranda et al. (2018) found the prevalence of *D. fragilis* to be associated with having children in the

home. Beyond parenthood, this may also extend to those who work in jobs such as child care, which tends to be dominated by females (van Polanen et al., 2017).

Given that the gut microbiota is a highly complex ecosystem in which protozoan members cohabitate and interact with other constituents such as bacteria (e.g. Burgess et al., 2017; Leung et al., 2018), it is difficult to comment exclusively on the effect of protozoa on symptom severity. As such, moderation analyses were conducted to determine whether bacteria (at the phylum level) moderated the relationship between protozoan carriage and psychological symptom severity. The Firmicutes and Bacteroidetes phyla were selected as moderating variables as these two phyla alone represent approximately 90% of gut microbiota (e.g. Rinninella et al., 2019). While phylum level data lacks the specificity of genus or species level data, the ratio of Firmicutes to Bacteroidetes continues to be used as a general measure of bacterial composition (e.g. Durk et al., 2019; Stojanov et al., 2020; Vaiserman et al., 2020). The results of the current study revealed that the level of either Firmicutes or Bacteroidetes did not moderate the relationship between protozoan carriage and psychological symptom severity across any of the four psychological symptom domains for males or females. This suggests that the effect of protozoan carriage was not altered by either phyla. It was beyond the scope of this paper to investigate interactions between protozoa and bacteria at the more specific genera and species levels, however this should be addressed in future studies.

The limitations of the current study are mainly related to the restricted characterisation of the sample given its retrospective and cross-sectional nature. Useful information regarding protozoan load, duration of carriage, and formal diagnostic criteria of patients were not collected, and therefore unknown. Additionally, lifestyle factors such as diet (and others), and medication use which are known to influence overall gut ecology were not available. Not having this information available made it impossible to control for these factors or to analyse their potentially important interactions with *Blastocystis* sp. and/or *D. fragilis*. Additionally, due to the retrospective and cross-sectional nature of the data collection, determining the possible mechanisms of action of these protozoa was not possible. The retrospective data also limited analysis due to the cross-sectional observance of intestinal protozoa within the sample. In line with previous descriptions (see Barratt et al., 2011) *D. fragilis* was far less common in males in the current study which did not allow for meaningful comparisons between groups, or in fact within the male sample at all. One contributing factor to this is the highly variable day to day shedding of the protozoan requiring multiple sampling and analysis of freshly passed stool, potentially meaning that the presence of *D. fragilis* is generally underestimated in cross sectional research (Peek et al., 2004; Stark et al., 2016). With regard to *Blastocystis* sp., although 10 subtypes have been identified in humans, only ST1 and ST3 were distinguished from *Blastocystis* ST unspecified. Therefore, it was unknown which specific *Blastocystis* STs the majority of those who tested positive for the protozoan were carrying. While this was somewhat circumvented by combining the *Blastocystis* STs to analyse the protozoan at the genus level, the findings therefore lack specificity.

A further limitation is that PCR testing only indicates the presence of protozoa, but does not provide information on whether the organisms are dead or alive (Cangelosi & Meschke, 2014). Therefore, it is possible that while individuals may have tested positive for *Blastocystis* sp. or *D. fragilis*, these organisms may have been dead and therefore not exerting their typical influence. Despite this, PCR testing is thought to be best practice for detecting intestinal protozoa (De Canale et al., 2009; Garcia et al., 2018; Padukone et al., 2018). Roberts et al. (2011) suggest that the results of studies that rely on microscopy alone should be interpreted with caution. It may be that differences in diagnostic techniques contribute to the controversy surrounding the relationship between intestinal protozoa and symptomatology due to the underestimation of intestinal protozoa.

Despite these limitations, the current study is the first to investigate differences in psychological symptom expression related to *Blastocysitis* and/or *D. fragilis* carriage, acting as an important first step for further research and elucidation of the possible influence of these protozoa on the holistic well-being of their human host. Future studies must make a concerted effort to address the aforementioned limitations. While it may currently be impossible to investigate the intricate multi-directional relationships within and beyond the gut, research in this area should endeavour to take the most holistic possible approach. For example, further studies should consider the possible moderating effects of specific bacteria on the relationship between protozoa and symptom expression. The possible moderating effect of protozoa on the relationship between bacteria and symptoms should also be considered.

Conclusion

The results of the current study add weight to the argument that *Blastocystis* sp. and *D. fragilis* are, potentially, commensal members of the human gut microbiota, and do not appear to contribute to self-reported psychological symptom severity. However, as this is the first study investing psychological symptoms, further research is required before solid conclusions can be drawn regarding the influence of these protozoa on psychological symptoms. Clinical trials investigating specific protozoan subtypes and multiple stool sampling to avoid underestimation of protozoan prevalence is warranted due to continued contradictory findings in relation to these protozoa and other symptom expressions (i.e. GI symptoms). Greater

research focus on these protozoa is required due to the tremendous inter- and intra-individual differences which may contribute to potential commensalism, symbiosis, or pathogenicity. With further research specifically focusing on the potential mechanisms of action of these protozoa, researchers will gain greater insight into, and understanding of, the variability in symptom expression of protozoan carriers. While the results of this study do not suggest that *Blastocystis* and *D. fragilis* should be used in the treatment of various symptomatologies, they do suggest that treatment of asymptomatic carriers of these protozoa should be cautiously considered, given the potential deleterious side effects of such treatments. The role that *Blastocystis* sp. and *D. fragilis* play in symptom expression has received comparatively less attention to the bacterial component of the gut microbiota, especially in regard to psychological symptom expression. As such, this paper serves as a call to action for BGMA researchers to consider protozoan members of the gut microbiota going forward to fill this glaring gap in the literature.

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Data Availability The data that support the findings of this study are available from Bioscreen but restrictions apply to the availability of these data, which were used under licence for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Bioscreen.

Declarations

Conflict of Interest The authors report no conflict of interest.

References

Abu-Madi, M. A., Behnke, J. M., Boughattas, S., Al-Thani, A., & Doiphode, S. H. (2016). A decade of intestinal protozoan epidemiology among settled immigrants in Qatar. *BMC Infectious Diseases, 16*, 370–370. https://doi.org/10.1186/s12879-016-1728-3.

Akoglu, H. (2018). User's guide to correlation coefficients. *Turkish Journal of Emergency Medicine, 18*, 91–93. https://doi.org/10.1016/j.tjem.2018.08.001.

Al-Hindi, A., & Shammala, B. M. A. (2013). Dientamoeba fragilis in Gaza Strip: a neglected protozoan parasite. *Iranian Journal of Parasitology, 8*(2), 249–255.

Altemus, M., Sarvaiya, N., & Neill Epperson, C. (2014). Sex differences in anxiety and depression clinical perspectives. *Frontiers in Neuroendocrinology, 35*(3), 320–330. https://doi.org/10.1016/j.yfrne.2014.05.004.

Audebert, C., Even, G., Cian, A., El Safadi, D., Certad, G., Delhaes, L., Pereira, B., Nourrisson, C., Poirier, P., Wawrzyniak, I., Delbac, F., Morelle, C., Bastien, P., Lachaud, L., Bellanger, A., Botterel, F., Candolfi, E., Desoubeaux, G., Morio, F., Pomares, C., Rabodonirina, M., Loywick, A., Merlin, S., Viscogliosi, E., & Chabé, M. (2016). Colonization with the enteric protozoa Blastocystis is associated with increased diversity of human gut bacterial microbiota. *Scientific Reports, 6*, 25255. https://doi.org/10.1038/srep25255.

Aykur, M., Caliskan Kurt, C., Dirim Erdogan, D., Biray Avci, C., Vardar, R., Aydemir, S., Girginkardeşler, N., Gündüz, C., & Dagci, H. (2019). Investigation of Dientamoeba fragilis prevalence and evaluation of Sociodemographic and clinical features in patients with gastrointestinal symptoms. *Acta Parasitologica, 64*(1), 162–170. https://doi.org/10.2478/s11686-018-00017-5.

Barratt, J. L., Harkness, J., Marriott, D., Ellis, J. T., & Stark, D. (2011). A review of Dientamoeba fragilis carriage in humans: Several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. *Gut Microbes, 2*(1), 3–12. https://doi.org/10.4161/gmic.2.1.14755.

Barsky, A. J., Peekna, H. M., & Borus, J. F. (2001). Somatic symptom reporting in women and men. *Journal of General Internal Medicine, 16*(4), 266–275.

Bart, A., Wentink-Bonnema, E. M., Gilis, H., Verhaar, N., Wassenaar, C. J., van Vugt, M., Goorhuis, A., & van Gool, T. (2013). Diagnosis and subtype analysis of Blastocystis sp. in 442 patients in a hospital setting in the Netherlands. *BMC Infectious Diseases, 13*, 389. https://doi.org/10.1186/1471-2334-13-389.

Beasley, T. M., & Schumacker, R. E. (1995). Multiple regression approach to analyzing contingency tables: Post hoc and planned comparison procedures. *The Journal of Experimental Education, 64*(1), 79–93. https://doi.org/10.1080/00220973.1995.9943797.

Beghini, F., Pasolli, E., Truong, T. D., Putignani, L., Cacciò, S. M., & Segata, N. (2017). Large-scale comparative metagenomics of Blastocystis, a common member of the human gut microbiome. *The ISME Journal, 11*, 2848–2863. https://doi.org/10.1038/ismej.2017.139.

Beiromvand, M., Hashemi, S. J., Arjmand, R., Sadjadei, N., & Hardanipasand, L. (2017). Comparative prevalence of Blastocystis in patients with the irritable bowel syndrome and healthy individuals: A case control study. *Jundishapur Journal of Microbiology, 10*(6), e13572. https://doi.org/10.5812/jjm.13572.

Boccabella, A., & Malouf, J. (2017). How do sleep-related health problems affect functional status according to sex? *Journal of Clinical Sleep Medicine, 13*(5), 685–692. https://doi.org/10.5664/jcsm.6584.

Brands, M. R., Van de Vijver, E., Haisma, S. M., Heida, A., & van Rheenen, P. F. (2019). No association between abdominal pain and Dientamoeba in Dutch and Belgian children. *Archives of Disease in Childhood*, archdischild-2018-316383. https://doi.org/10.1136/archdischild-2018-316383.

Bruce-Keller, A. J., Salbaum, J. M., & Berthoud, H. R. (2018). Harnessing gut microbes for mental health: Getting from Here to there. *Biological Psychiatry, 83*(3), 214–223. https://doi.org/10.1016/j.biopsych.2017.08.014.

Burgess, S. L., Gilchrist, C. A., Lynn, T. C., & Petri, W. A. (2017). Parasitic Protozoa and interactions with the host intestinal microbiota. *Infection and Immunity, 85*(8), e00101–e00117. https://doi.org/10.1128/IAI.00101-17.

Butler, W. P. (1996). Dientamoeba fragilis. An unusual intestinal pathogen. *Digestive Diseases and Sciences, 41*(9), 1811–1813. https://doi.org/10.1007/bf02088750.

Cangelosi, G. A., & Meschke, J. S. (2014). Dead or alive: Molecular assessment of microbial viability. *Applied and Environmental Microbiology, 80*(19), 5884–5891. https://doi.org/10.1128/aem.01763-14.

Chabé, M., Lokmer, A., & Segurel, L. (2017). Gut Protozoa: Friends or foes of the human gut microbiota? *Trends in Parasitology, 33*(12), 925–934. https://doi.org/10.1016/j.pt.2017.08.005.

Chandramathi, S., Suresh, K., Anita, Z. B., & Kuppusamy, U. R. (2012). Infections of Blastocystis hominis and microsporidia in cancer patients: Are they opportunistic? *Transactions of the Royal Society of Tropical Medicine and Hygiene, 106*(4), 267–269. https://doi.org/10.1016/j.trstmh.2011.12.008.

Chandramathi, S., Suresh, K., Sivanandam, S., & Kuppusamy, U. R. (2014). Stress exacerbates infectivity and pathogenicity of Blastocystis hominis: In vitro and in vivo evidences. *PLoS One, 9*(5), e94567–e94567. https://doi.org/10.1371/journal.pone.0094567.

Chudnovskiy, A., Mortha, A., Kana, V., Kennard, A., Ramirez, J. D., Rahman, A., Remark, R., Mogno, I., Ng, R., Gnjatic, S., Amir, E. A. D., Solovyov, A., Greenbaum, B., Clemente, J., Faith, J., Belkaid, Y., Grigg, M. E., & Merad, M. (2016). Host-protozoan interactions protect from mucosal infections through activation of the inflammasome. *Cell, 167*(2), 444–456. https://doi.org/10.1016/j.cell.2016.08.076.

Cohen, J. (1969). *Statistical power analysis for the behavioural sciences*. Academic Press.

Cohen, J., Cohen, P., West, S. G., & Aiken, L. S. (2013). *Applied multiple regression/correlation analysis for the behavioural sciences (3rd ed.)*. Lawrence Erlbaum Associates Publishers.

Coulson, S., Butt, H., Vecchio, P., Gramotnev, H., & Vietta, L. (2013). Green-lipped mussel extract (Perna canaliculus) and glucosamine sulphate in patients with knee osteoarthritis: Therapeutic efficacy and effects on gastrointestinal microbiota profiles. *Inflammopharmacology, 21*, 79–90.

Coyle, C. M., Varughese, J., Weiss, L. M., & Tanowitz, H. B. (2011). Blastocystis: To treat or not to treat…. *Clinical Infectious Diseases, 54*(1), 105–110. https://doi.org/10.1093/cid/cir810.

Crotti, D., & D'Annibale, M. L. (2007). Human intestinal parasitosis: Role of Dientamoeba fragilis in human infections. *Annali di Igiene, 19*(1), 27–34.

Dawkins, R., & Krebs, J. R. (1979). Arms race between and within species. *Proceedings of the Royal Society of London. Series B, Biological Sciences, 205*(1161), 489–511.

De Canale, E., Biasolo, M. A., Tessari, A., Bettanello, S., Besutti, V., & Mengoli, C. (2009). Real time PCR for Dientamoeba fragilis: A comparison between molecular and microscopical approach. *Microbiologia Medica, 24*(3). https://doi.org/10.4081/mm.2009.2524.

Deng, L., Wojciech, L., Gascoigne, N. R. J., Peng, G., & Tan, K. S. W. (2021). New insights into the interactions between Blastocystis, the gut microbiota, and host immunity. *PLoS Pathogens, 17*(2), e1009253. https://doi.org/10.1371/journal.ppat.1009253.

Dunwell, D. (2013). ME/CFS and Blastocystis spp or Dientamoeba fragilis, an in-house comparison. *British Journal of General Practice, 63*(607), 73–74. https://doi.org/10.3399/bjgp13X663028.

Durk, R. P., Castillo, E., Márquez-Magaña, L., Grosicki, G. J., Bolter, N. D., Lee, C. M., & Bagley, J. R. (2019). *International Journal of Sport Nutrition and Exercise Metabolism, 29*(3), 249–253. https://doi.org/10.1123/ijsnem.2018-0024.

El Aidy, S., Stilling, R., Dinan, T. G., & Cryan, J. F. (2016). Microbiome to brain: Unravelling the multidirectional axes of communication. *Advances in Experimental Medicine and Biology, 874*, 301–336. https://doi.org/10.1007/978-3-319-20215-0_15.

El-Zawawy, H. T., Farag, H. F., Tolba, M. M., & Abdalsamea, H. A. (2020). Improving Hashimoto's thyroiditis by eradicating *Blastocyistis hominis*: Relation to IL-17. *Theraputic Advances in Endocrinology and Metabolism, 11*, 1–8. https://doi.org/10.1177/2042018820907013.

Flak, M. B., Neves, J. F., & Blumberg, R. S. (2013). Immunology. Welcome to the microgenderome. *Science, 339*(6123), 1044–1045. https://doi.org/10.1126/science.1236226.

Garcia, L. S. (2016). Dientamoeba fragilis; one of the neglected intestinal Protozoa. *Journal of Clinical Microbiology, 54*(9), 2243–2250. https://doi.org/10.1128/JCM.00400-16.

Garcia, L. S., Arrowood, M., Kokoskin, E., Paltridge, G. P., Pillai, D. R., Procop, G. W., Ryan, N., Shimizu, R. Y., & Visvesvara, G. (2018). Laboratory diagnosis of parasites from the gastrointestinal tract. *Clinical Microbiology Reviews, 31*(1), e00025–e00017. https://doi.org/10.1128/CMR.00025-17.

Giloteaux, L., Goodrich, J. K., Walters, W. A., Levine, S. M., Ley, R. E., & Hanson, M. R. (2016). Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome, 4*(1), 30. https://doi.org/10.1186/s40168-016-0171-4.

Grendon, J. H., DiGiacomo, R. F., & Frost, F. J. (1995). Descriptive features of Dientamoeba fragilis infections. *The Journal of Tropical Medicine and Hygiene, 98*(5), 309–315.

Hall, E., & Steiner, M. (2013). Serotonin and female psychopathology. *Women's Health, 9*(1), 85–97. https://doi.org/10.2217/WHE.12.64.

Hamidi, N., Meamar, A. R., Akhlaghi, L., Rampisheh, Z., & Razmjou, E. (2018). Dientamoeba fragilis diagnosis be fecal screening: Relative effectiveness of traditional techniques and molecular methods. *The Journal of Infection in Developing Countries, 12*(1), 52–59. https://doi.org/10.3855/jidc.9643.

Hayes, A. F. (2017). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. Guilford publication.

Ho, J. M. W., & Juurlink, D. N. (2011). Considerations when prescribing trimethoprim-sulfamethoxazole. *Canadian Medical Association*

Journal, 183(16), 1851–1858. https://doi.org/10.1503/cmaj.111152.

Holtman, G. A., Kranenberg, J. J., Blanker, M. H., Ott, A., Lisman-van Leeuwen, Y., & Berger, M. Y. (2017). Dientamoeba fragilis colonization is not associated with gastrointestinal symptoms in children at primary care level. *Family Practice, 34*(1), 25–29. https://doi.org/10.1093/fampra/cmw111.

Huberty, C. J, & Petoskey, M. D. (2000). Multivariate analysis of variance and covariance. In H. E. A. Tinsley & S. D. Brown (Eds.), Handbook of applied multivariate statistics and mathematical modeling (p. 183–208). Academic Press.https://doi.org/10.1016/B978-012691360-6/50008-2

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., & Ruan, B. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, Behaviour, and Immunity, 48*, 186–194. https://doi.org/10.1016/j.bbi.2015.03.016.

Jiménez, P. A., Jaimes, J. E., & Ramírez, J. D. (2019). A summary of Blastocystis subtypes in north and South America. *Parasites & Vectors, 12*(1), 376. https://doi.org/10.1186/s13071-019-3641-2.

Johnson, E. H., Windsor, J. J., & Clark, C. G. (2004). Emerging from obscurity: Biological, clinical, and diagnostic aspects of Dientamoeba fragilis. *Clinical Microbiology Review, 17*(3), 553–570. https://doi.org/10.1128/CMR.17.3.553-570.2004.

Jokelainen, P., Hebbelstrup Jensen, B., Andreassen, B. U., Petersen, A. M., Roser, D., Krogfelt, K. A., Nielsen, H. V., & Stensvold, C. R. (2017). Dientamoeba fragilis, a commensal in children in Danish day care centers. *Journal of Clinical Microbiology, 55*(6), 1707–1713. https://doi.org/10.1128/jcm.00037-17.

Kaneda, Y., Horiki, N., Cheng, X., Tachibana, H., & Tsutsumi, Y. (2000). Serologic response to Blastocystis hominis infection in asymptomatic individuals. *The Tokai Journal of Experimental and Clinical Medicine, 25*(2), 51–56.

Kesuma, Y., Firmansyah, A., Bardosono, S., Sari, I. P., & Kurniawan, A. (2019). Blastocystis ST-1 is associated with irritable bowel sundrom-diarrhoea (ISB-D) in Indonesian adolescence. *Parasite Epidemiology and Control, 6*, 1–9. https://doi.org/10.1016/j.parepi.2009.e00112.

Klein, S. L. (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology, 26*(6–7), 247–264. https://doi.org/10.1111/j.0141-9838.2004.00710.x.

Kovats, S. (2015). Estrogen receptors regulate innate immune cells and signaling pathways. *Cellular Immunology, 294*(2), 63–69. https://doi.org/10.1016/j.cellimm.2015.01.018.

Krogsgaard, L. R., Engsbro, A. L., Stensvold, C. R., Nielsen, H. V., & Bytzer, P. (2015). The prevalence of intestinal parasites is not greater among individuals with irritable bowel syndrome: A population-based case-control study. *Clinical Gastroenterology and Hepatology, 13*(3), 507–513.e502. https://doi.org/10.1016/j.cgh.2014.07.065.

Krogsgaard, L. R., Andersen, L. O. B., Johannesen, T. B., Engsbro, A. L., Stensvold, C. R., Nielsen, H. V., & Bytzer, P. (2018). Characteristics of the bacterial microbiome in association with common intestinal parasites in irritable bowel syndrome. *Clinical and Translational Gastroenterology, 9*(6), 161–161. https://doi.org/10.1038/s41424-018-0027-2.

Laanto, E., Hoikkala, V., Ravantti, J., & Sundberg, L. R. (2017). Long-term genomic coevolution of host-parasite interaction in the natural environment. *Nature Communications, 8*, 1–8. https://doi.org/10.1038/s41467-017-00158-7.

Lepczyńska, M., Białkowska, J., Dzika, E., Piskorz-Ogórek, K., & Korycińska, J. (2017). Blastocystis: How do specific diets and human gut microbiota affect its development and pathogenicity? *European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology, 36*(9), 1531–1540. https://doi.org/10.1007/s10096-017-2965-0.

Leung, J. M., Graham, A. L., & Knowles, S. C. (2018). Parasite-microbiota interaction with the vertebrate gut: Synthesis through an ecological lens. *Frontiers in Microbiology, 9*(843), 1–20. https://doi.org/10.3389/fmic.2018.00843.

Li, Q., Han, Y., Dy, A. B. C., & Hagerman, R. J. (2017). The gut microbiota and autism Spectrum disorders. *Frontiers in Cellular Neuroscience, 11*, 120–120. https://doi.org/10.3389/fncel.2017.00120.

Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine, 8*(1), 51. https://doi.org/10.1186/s13073-016-0307-y.

Lokmer, A., Cian, A., Froment, A., Gantois, N., Viscogliosi, E., Chabé, M., & Ségurel, L. (2019). Use of shotgun metagenomics for the identification of protozoa in the gut microbiota of healthy individuals from worldwide populations with various industrialization levels. *PLoS One, 14*(2), e0211139. https://doi.org/10.1371/journal.pone.0211139.

Lukeš, J., Stensvold, C. R., Jirků-Pomajbíková, K., & Wegener Parfrey, L. (2015). Are human intestinal eukaryotes beneficial or commensals? *PLoS Pathogens, 11*(8), e1005039. https://doi.org/10.1371/journal.ppat.1005039.

MacLean, A., Sweeting, H., & Hunt, K. (2010). 'Rules' for boys, 'guidelines' for girls: Gender differences in symptom reporting during childhood and adolescence. *Social Science and Medicine, 70*(4), 597–604. https://doi.org/10.1016/j.socschimedi.2009.10.042.

Maeng, L. Y., & Milad, M. R. (2015). Sex differences in anxiety disorders: Interactions between fear, stress, and gonadal hormones. *Hormones and Behaviour, 76*, 106–117. https://doi.org/10.1016/j.yhbeh.2015.04.002.

Maizels, R. M. (2009). Parasite immunomodulation and polymorphisms of the immune system. *Journal of Biology, 8*(62), 1–4. https://doi.org/10.1186/jbiol166.

Markle, J. G., Frank, D. N., Mortin-Toth, S., Robertson, C. E., Feazel, L. M., Rolle-Kampczyk, U., von Bergen, M., McCoy, K. D., Macpherson, A. J., & Danska, J. S. (2013). Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science, 339*(6123), 1084–1088. https://doi.org/10.1126/science.1233521.

Marriott, I., & Huet-Hudson, Y. M. (2006). Sexual dimorphism in innate immune responses to infectious organisms. *Immunological Research, 34*(3), 177–192. https://doi.org/10.1385/ir:34:3:177.

McHugh, M. L. (2013). The chi-square test of independence. *Biochemica Medica, 23*(2), 143–149. https://doi.org/10.11613/BM.2013.018.

Menendez Fernandez-Miranda, C., Fernandez-Suarez, J., Rodriguez-Perez, M., Menendez Fernandez-Miranda, P., Vazquez, F., Boga Ribeiro, J. A., & Rodriguez-Guardado, A. (2018). Prevalence of D. fragilis infection in the household contacts of a group of infected patients. *Enfermedades Infecciosas y Microbiología Clínica, 36*(7), 423–427. https://doi.org/10.1016/j.eimc.2017.08.004.

Mulak, A., Taché, Y., & Larauche, M. (2014). Sex hormones in the modulation of irritable bowel syndrome. *World Journal of Gastroenterology, 20*(10), 2433–2448. https://doi.org/10.3748/wjg.v20.i10.2433.

Nagata, N., Marriott, D., Harkness, J., Ellis, J. T., & Start, D. (2012). Current treatment options for Dientamoeba fragilis infections. *The International Journal for Parasitology – Drugs and Drug Resistance, 2*, 204–215. https://doi.org/10.1016/j.ijpddr.2012.08.002.

Norberg, A., Nord, C. E., & Evengard, B. (2003). Dientamoeba fragilis–a protozoal infection which may cause severe bowel distress. *Clinical Microbiology and Infection, 9*(1), 65–68.

Nourrisson, C., Scanzi, J., Pereira, B., NkoudMongo, C., Wawrzyniak, I., Cian, A., Viscogliosi, E., Livrelli, V., Delbec, F., Dapoigny, M., & Poirier, P. (2014). Blastocystis is associated with decrease of fecal microbiota protective bacteria: Comparative analysis between patients with irritable bowel syndrome and control subjects. *PLoS One, 9*(11), e111868. https://doi.org/10.1371/journal.pone.0111868.

Padukone, S., Mandal, J., Rajkumari, N., Bhat, B., Swaminathan, R., & Parija, S. (2018). Detection of *Blastocystis* in clinical stool specimens using three different methods and morphological examination in Jones' medium. *Tropical Parasitology, 8*(1), 33–40. https://doi.org/10.4103/tp.TP_4_18.

Pavanelli, M. F., Kaneshima, E. N., Uda, C. F., Colli, C. M., Falavigna-Guilherm, A. L., & Gomes, M. L. (2015). Pathogenicity of Blastocystis sp. to the gastrointestinal tract of mice: Relationship between inoculum size and period of infection. *Revista do Instituto de Medicina Tropical de Sao Paulo, 57*(6), 467–472. https://doi.org/10.1590/S0036-46652015000600002.

Peek, R., Reedeker, F. R., & van Gool, T. (2004). Direct amplification and genotyping of Dientamoeba fragilis from human stool specimens. *Journal of Clinical Microbiology, 42*(2), 631–635. https://doi.org/10.1128/jcm.42.2.631-635.2004.

Qadri, S. M., al-Okaili, G. A., & al-Dayel, F. (1989). Clinical significance of Blastocystis hominis. *Journal of Clinical Microbiology, 27*(11), 2407–2409.

Rea, K., Dinan, T. G., & Cryan, J. F. (2016). The microbiome: A key regulator of stress and neuroinflammation. *Neurobiology of Stress, 4*, 23–33. https://doi.org/10.1016/j.ynstr.2016.03.001.

Rhoads, S. E., & Rhoads, C. H. (2012). Gender roles and infant/toddler care: Male and female professors on the tenure track. *Journal of Social, Evolutionary, and Cultural Psychology, 6*(1), 13–31. https://doi.org/10.1037/h0099227.

Richardson, J. T. E. (2011). Eta squared and partial eta squared as measures of effect size in educational research. *Educational Research Review, 6*(2), 135–147. https://doi.org/10.1016/j.edurev.2010.12.001.

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., & Mele, M. C. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms, 7*(1), 14. https://doi.org/10.3390/microorganisms7010014.

Roberts, T., Barratt, J., Harkness, J., Ellis, J., & Stark, D. (2011). Comparison of microscopy, culture, and conventional polymerase chain reaction for detection of blastocystis sp. in clinical stool samples. *The American Journal of Tropical Medicine and Hygiene, 84*(2), 308–312. https://doi.org/10.4269/ajtmh.2011.10-0447.

Rook, G. A. (2013). Regulation of the immune system by biodiversity from the natural environment: An ecosystem service essential to health. *Proceedings of the National Academy of Sciences of the United States of America, 110*(46), 18360–18367. https://doi.org/10.1073/pnas.1313731110.

Rook, G. A., & Brunet, L. R. (2005). Microbes, immunoregulation, and the gut. *Gut, 54*(3), 317–320. https://doi.org/10.1136/gut.2004.053785.

Rook, G. A., Raison, C. L., & Lowry, C. A. (2014). Microbial 'old friends', immunoregulation and socioeconomic status. *Clinical and Experimental Immunology, 177*(1), 1–12. https://doi.org/10.1111/cei.12269.

Röser, D., Simonsen, J., Nielsen, H. V., Stensvold, C. R., & Mølbak, K. (2013). Dientamoeba fragilis in Denmark: Epidemiological experience derived from four years of routine real-time PCR. *European Journal of Clinical Microbiology & Infectious Diseases, 32*(10), 1303–1310. https://doi.org/10.1007/s10096-013-1880-2.

Rossen, N. G., Bart, A., Verhaar, N., van Nood, E., Kootte, R., de Groot, P. F., D'Haens, G. R., Ponsioen, C. Y., & van Gool, T. (2015). Low prevalence of Blastocystis sp. in active ulcerative colitis patients. *European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology, 34*(5), 1039–1044. https://doi.org/10.1007/s10096-015-2312-2.

Salehi, M., Mardaneh, J., Niazkar, H. R., Minooeianhaghighi, M., Arshad, E., Soleimani, F., & Mohammadzadeh, A. (2021). Prevalence and subtype analysis of Blastocystis hominis isolated from patients in the northeast of Iran. *Journal of Parasitology Research, 2021*, 1–8. https://doi.org/10.1155/2021/8821885.

Scanlan, P. D., Stensvold, C. R., Rajilić-Stojanović, M., Heilig, H. G. H. J., De Vos, W. M., O'Toole, P. W., & Cotter, P. D. (2014). The microbial eukaryote Blastocystis is a prevalent and diverse member of the healthy human gut microbiota. *FEMS Microbiology Ecology, 90*(1), 326–330. https://doi.org/10.1111/1574-6941.12396.

Seney, M. L., & Sibille, E. (2014). Sex differences in mood disorders: Perspectives from humans and rodent models. *Biology of Sex Differences, 5*(1), 17–17. https://doi.org/10.1186/s13293-014-0017-3.

Seyer, A., Karasartova, D., Ruh, E., Güreser, A. S., Turgal, E., Imir, T., & Taylan-Ozkan, A. (2017). Epidemiology and prevalence of Blastocystis spp. in North Cyprus. *The American Journal of Tropical Medicine and Hygiene, 96*(5), 1164–1170. https://doi.org/10.4269/ajtmh.16-0706.

Solomon, M. B., & Herman, J. P. (2009). Sex differences in psychopathology: Of gonads, adrenals and mental illness. *Physiology & Behaviour, 97*(2), 250–258. https://doi.org/10.1016/j.physbeh.2009.02.033.

Sorge, R. E., & Strath, L. J. (2018). Sex differences in pain responses. *Current Opinion in Physiology, 6*, 75–81. https://doi.org/10.1016/j.cophys.2018.05.006.

Sorge, R. E., & Totsch, S. K. (2017). Sex differences in pain. *Journal of Neuroscience Research, 95*(6), 1271–1281. https://doi.org/10.1002/jnr.23841.

Stark, D., Barratt, J., Roberts, T., Marriott, D., Harkness, J., & Ellis, J. (2010). A review of the clinical presentation of dientamoebiasis. *The American Journal of Tropical Medicine and Hygiene, 82*(4), 614–619. https://doi.org/10.4269/ajtmh.2010.09-0478.

Stark, D., Barratt, J., Chan, D., & Ellis, J. T. (2016). Dientamoeba fragilis, the neglected trichomonad of the human bowel. *Clinical*

Microbiology Reviews, 29(3), 553–580. https://doi.org/10.1128/CMR.00076-15.

Stensvold, C. R., & Clark, C. G. (2016). Current status of Blastocystis: A personal view. *Parasitol Int, 65*(6 Pt B), 763–771. https://doi.org/10.1016/j.parint.2016.05.015.

Stojanov, S., Berlec, A., & Štrukelj, B. (2020). The influence of probiotics on the Firmicutes/Bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. *Microorganisms, 8*(1715), 1–16. https://doi.org/10.3390/microorganisms8111715.

Taneja, V. (2018). Sex Hormones Determine Immune Response. *Frontiers in Immunology, 9*, 1931–1931. https://doi.org/10.3389/fimmu.2018.01931.

Tito, R. Y., Chaffron, S., Caenepeel, C., Lima-Mendez, G., Wang, J., Vieira-Silva, S., Falony, G., Hildebrand, F., Darzi, Y., Rymenans, L., Verspecht, C., Bork, P., Vermeire, S., Joossens, M., & Raes, J. (2019). Population-level analysis of *Blastocystis* subtype prevalence and variation in the human gut microbiota. *Gut, 68*(7), 1180–1189. https://doi.org/10.1136/gutjnl-2018-316106.

Tsaousis, A. D., Hamblin, K. A., Elliott, C. R., Young, L., Rosell-Hidalgo, A., Gourlay, C. W., Moore, A. L., & van der Giezen, M. (2018). The human gut colonizer Blastocystis respires using complex II and alternative oxidase to buffer transient oxygen fluctuations in the gut. *Frontiers in Cellular and Infection Microbiology, 8*, 371–371. https://doi.org/10.3389/fcimb.2018.00371.

Vaiserman, A., Romanenko, M., Piven, L., Moseiko, V., Lushchak, O., Kryzhanovska, N., Guryanov, V., & Koliada, A. (2020). Differences in the gut Firmicutes and Bacteroidetes ratio across age groups in healthy Ukrainian population. *BMC Microbiology, 20*, 1–8. https://doi.org/10.1186/s12866-020-01903-7.

Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *BMJ, 361*, k2179. https://doi.org/10.1136/bmj.k2179.

van Polanen, M., Colonnesi, C., Tavecchio, L. W. C., Blokhuis, S., & Fukkink, R. G. (2017). Men and women in childcare: A study of caregiver–child interactions. *European Early Childhood Education Research Journal, 25*(3), 412–424. https://doi.org/10.1080/1350293X.2017.1308165.

Vemuri, R., Sylvia, K. E., Klein, S. L., Forster, S. C., Plebanski, M., Eri, R., & Flanagan, K. L. (2019). The microgenderome revealed: Sex differences in bidirectional interactions between the microbiota, hormones, immunity and disease susceptibility. *Seminars in Immunopathology, 41*(2), 265–275. https://doi.org/10.1007/s00281-018-0716-7.

Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., Carlsson, C. M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B. B., & Rey, F. E. (2017). Gut microbiome alterations in Alzheimer's disease. *Scientific Reports, 7*(1), 13537. https://doi.org/10.1038/s41598-017-13601-y.

Wallis, A., Butt, H., Ball, M., Lewis, D. P., & Bruck, D. (2016). Support for the Microgenderome: Associations in a human clinical population. *Scientific Reports, 6*, 19171. https://doi.org/10.1038/srep19171.

Weir, C. B. & Le, J. K. (2020). Metronidazole. In: StatPearls (internet). StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539728/

Yadav, R. K., Malhotra, S., & Duggal, N. (2020). Immune dysfunction during enteric protozoal infection: The current trends. In S. K. Saxena & H. Prakash *Innate Immunity in Health and Disease*. IntechOpen. https://doi.org/10.5772/intechopen.91698.

Zuk, M. (2009). The Sicker Sex. *PLOS Pathogens, 5*(1), e1000267. https://doi.org/10.1371/journal.ppat.1000267.

Zulfa, F., Sari, I. P., & Kurniawan, A. (2017). Association of Blastocystis subtypes with diarrhea in children. *Journal of Physics: Conference Series, 884*, 1–6.

Paper 2 Supplemental Material (Online Resource 1): Exploring the Validity of the Bioscreen Patient Questionnaire

The symptom severity items of the Bioscreen patient questionnaire (BPQ) were subjected to exploratory factor analysis (EFA) using the maximum likelihood (ML) method of extraction with an oblique (direct oblimin) rotation. The ML method of extraction was chosen given that the aim was to reveal latent variables underlying the data. This differs from principal components analysis which is primarily a data reduction method which does not discriminate between shared and unique variance (Costello & Osbourne, 2005).

Of the 88 original items, four items were removed prior to analysis. These items included three sex specific symptoms ("ovulation or menstruation pain", "vaginal irritation or discomfort", and "orchialgia or testicular pain"). Additionally, an item referring to "sore or swollen lymph glands" was removed due to its similarity to items "sore or swollen lymph glands in the neck" and "sore or swollen lymph glands in the groin", which were retained. The remaining 84 items were subjected to EFA in a sample of 3290 participants (following listwise deletion) using SPSS version 25.

Loadings below .3 were supressed (Pallant, 2016). Items that did not load onto any factor were removed one at a time, with the analysis being repeated until all items loaded onto at least one factor. Only the final analysis in which all items loaded onto at least one factor are discussed from this point.

Using the Kaiser criterion, 10 factors were identified as having eigenvalues above 1, as can be seen in Table 1. While the Kaiser criterion has been criticised for retaining too many factor (over factoring), Catell's scree test suggests using either a two or three factor model, as can be seen in Figure 1. This appears to have had the opposite effect where too few factors are specified (under factoring). According to Fabrigar et al. (1999), under factoring is typically regarded as being a much more severe problem than over factoring. Additionally, the 10 factors suggested using the Kaiser

criterion appear to be clinically meaningful, whereas both the two and three factor models appear to be a combination of distinct factors, a symptom of under factoring (Fabrigar et al., 1999).

Table 1.

Eigenvalues, explained variance, and cumulative variance as a result of EFA

Factor	Eigenvalue	Variance explained (%)	Cumulative variance (%)
1	18.10	34.14	34.14
2	3.44	6.16	40.30
3	2.23	4.22	44.52
4	2.02	3.80	48.32
5	1.57	2.95	51.27
6	1.43	2.70	53.97
7	1.32	2.49	56.46
8	1.24	2.33	58.79
9	1.07	2.01	60.80
10	1.01	1.90	62.70

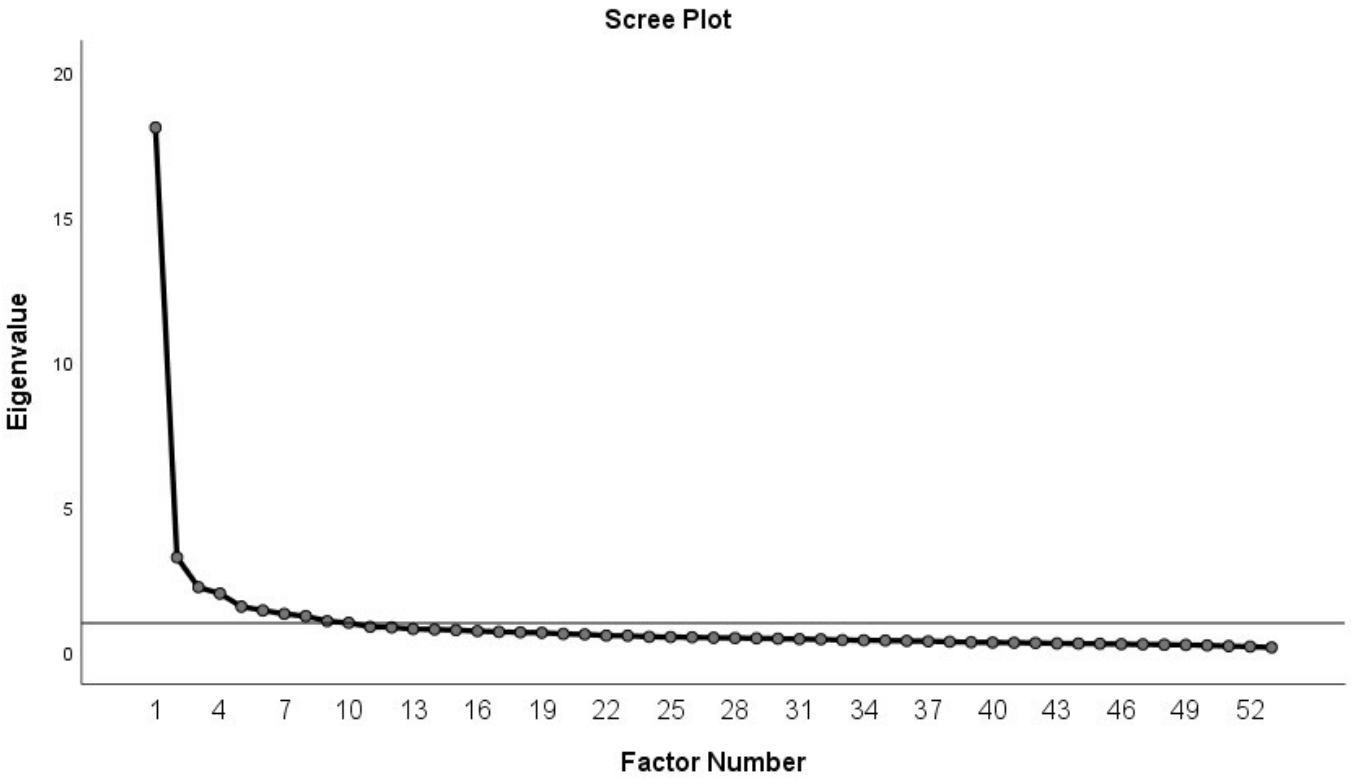

Figure 1. Scree plot for EFA

The full pattern matrix for the 10 extracted factors is presented in Table 2, with major loadings highlighted. As can be seen in Table 2., most of the items load cleanly onto only one factor, with minimal cross-loadings. The relationship between each item and the factor on which it loads can be seen in the structure matrix presented in Table 3.

Table 2.

Pattern matrix for EFA with direct oblimin rotation of ten factor solution of BPQ items

Item	Low mood (F1)	Pain (F2)	Neuro-cognition (F3)	Gastro-intestinal (F4)	Exertion (F5)	Urination (F6)	Neck / shoulder pain (F7)	Stress / anxiety (F8)	Sleep / fatigue (F9)	Immunity (F10)
3	**.694**	.006	-.018	.055	.069	.062	-.019	.083	.024	.016
20	**.371**	.003	-.009	.058	.020	.031	-.047	.238	.035	.053
28	**.656**	.035	-.063	.100	-.156	.024	-.043	-.073	.093	-.020
30	**.807**	.037	-.012	.033	-.009	.059	.001	.044	.039	.012
32	**.477**	.043	-.173	-.028	-.015	.023	.017	.077	.112	.063
54	**.501**	.018	-.056	-.018	-.117	.027	.020	.312	-.024	.026
18	.002	**.649**	-.026	.000	.038	.016	.055	-.002	-.050	.014
22	-.008	**.478**	-.017	.028	-.196	.026	-.137	.034	-.026	.048
24	-.002	**.751**	-.048	.026	.001	-.008	-.060	.000	.078	-.007
25	.035	**.819**	-.002	.003	-.007	.004	.005	.007	.057	.017
27	.031	**.336**	-.013	.136	.007	.003	-.239	.049	.055	.015

42	.004	**.355**	-.037	.064	-.195	.038	-.326	-.007	.087	-.007
9	.073	.061	**-.858**	-.023	.134	.011	-.048	-.128	.028	.021
38	.053	.024	**-.508**	.039	-.130	.060	.004	.079	-.007	.026
46	.144	-.041	**-.512**	-.011	.004	.007	-.011	.199	.043	.053
51	.015	.035	**-.802**	.019	-.031	.007	-.025	-.010	.013	.019
55	.091	-.052	**-.659**	.036	-.095	.023	-.003	.053	.114	-.004
62	-.006	.056	**-.880**	-.013	.038	.042	.022	.001	.009	.010
70	-.087	-.015	**-.654**	.039	-.094	.026	-.024	.097	-.022	.040
78	-.031	.003	**-.813**	.090	-.031	.014	.005	.092	.011	-.023
17	.034	-.050	-.073	**.532**	.024	-.015	-.064	-.054	.027	.017
23	.074	.084	.038	**.602**	.006	.003	-.088	-.021	.021	.035
37	-.043	.022	.006	**.551**	-.007	.026	.059	.016	-.025	.016
40	.061	.006	.038	**.567**	-.053	.037	-.027	.022	.038	.084
77	-.031	-.007	-.021	**.828**	.030	-.002	.041	.028	.009	-.074
48	-.018	.076	-.112	.038	**-.327**	.078	-.016	.054	.041	.156
50	.158	.161	-.022	.027	**-.544**	.071	-.022	-.035	.082	-.015

56	.061	.117	-.116	.047	**-.492**	.060	-.109	.002	.156	.035
58	.007	-.014	-.085	.029	**-.558**	.060	-.020	-.024	.211	.081
43	.033	-.025	.005	.017	.040	**.742**	-.041	-.035	.073	-.017
57	.002	-.007	.014	.099	-.079	**.341**	.017	.048	-.077	.089
63	-.009	.011	-.043	-.059	.028	**.868**	-.012	-.032	-.011	-.045
15	.009	-.025	-.024	-.004	.020	.041	**-.812**	.011	.015	.059
16	-.035	.031	.002	.002	.027	.035	**-.845**	.035	.005	-.015
35	.100	.048	-.024	-.027	-.010	.051	-.013	**.428**	.069	.045
36	.230	.017	-.062	-.011	-.128	.020	-.025	**.361**	.020	.064
72	.250	-.024	-.079	.069	-.071	.045	-.059	**.462**	-.094	.029
74	.028	.023	-.097	.016	.004	.032	.013	**.449**	-.001	.042
79	.151	.017	-.026	.050	-.135	.016	-.001	**.584**	.013	-.026
81	-.041	.093	-.013	-.007	-.005	.024	-.023	**.649**	.013	-.018
86	-.026	-.047	-.009	.039	.065	.006	-.054	**.456**	.085	.029
87	.232	-.050	-.073	.121	.036	.008	-.048	**.492**	.042	.014
88	.134	-.023	-.115	.044	.015	.023	-.033	**.519**	.039	-.010

14	.150	-.026	-.102	.068	-.167	-.032	-.113	-.102	**.536**	.043
34	.076	.045	-.078	.027	-.056	.005	-.030	-.001	**.658**	.048
44	.054	.068	.053	.069	.000	.101	-.007	.127	**.355**	.081
64	-.017	-.050	-.128	.040	-.049	.062	-.032	.074	**.463**	.054
66	.004	.104	.037	.062	-.013	.093	-.012	.176	**.452**	.041
69	.060	-.053	-.284	.086	-.207	-.016	-.045	.113	**.416**	-.019
2	-.009	.023	-.040	.042	.110	.017	-.053	-.020	.089	**.430**
31	.077	-.020	.032	.030	.004	-.021	-.022	-.034	-.031	**.755**
45	-.053	.047	-.004	-.037	-.014	.041	.055	.019	.031	**.452**
53	.033	-.052	-.058	.059	-.140	-.025	-.096	.032	-.065	**.510**

Table 3.

Structure matrix for EFA with direct oblimin rotation of ten factor solution of BPQ items

Item	Low mood (F1)	Pain (F2)	Neuro-cognition (F3)	Gastro-intestinal (F4)	Exertion (F5)	Urination (F6)	Neck / shoulder pain (F7)	Stress / anxiety (F8)	Sleep / fatigue (F9)	Immunity (F10)
3	**.787**	.167	-.421	.382	-.231	.326	-.266	.550	.367	.243
20	**.579**	.171	-.376	.345	-.221	.298	-.269	.531	.326	.267
28	**.785**	.267	-.500	.446	-.455	.347	-.355	.471	.469	.279
30	**.895**	.222	-.476	.409	-.336	.361	-.297	.589	.424	.270
32	**.664**	.223	-.518	.328	-.315	.324	-.277	.507	.437	.301
54	**.745**	.209	-.482	.340	-.374	.343	-.259	.666	.349	.276
18	.079	**.616**	-.160	.131	-.174	.204	-.240	.099	.080	.169
22	.215	**.644**	-.318	.285	-.435	.328	-.460	.229	.236	.321
24	.194	**.810**	-.322	.273	-.321	.312	-.462	.203	.288	.281
25	.202	**.845**	-.289	.250	-.327	.317	-.422	.203	.258	.284
27	.258	**.514**	-.318	.370	-.264	.301	-.499	.267	.302	.298

42	.290	**.631**	-.413	.386	-.482	.382	-.629	.270	.390	.355
9	.378	.277	**-.829**	.308	-.241	.342	-.354	.337	.426	.324
38	.411	.268	**-.672**	.351	-.403	.384	-.310	.431	.375	.335
46	.518	.190	**-.706**	.342	-.304	.354	-.304	.557	.428	.340
51	.416	.299	**-.859**	.369	-.382	.387	-.372	.427	.451	.366
55	.510	.231	**-.827**	.403	-.425	.401	-.350	.498	.529	.356
62	.401	.295	**-.891**	.340	-.331	.406	-.334	.438	.443	.354
70	.326	.240	**-.732**	.338	-.367	.362	-.321	.415	.367	.347
78	.441	.274	**-.891**	.428	-.383	.414	-.359	.508	.468	.359
17	.245	.124	-.279	**.570**	-.152	.236	-.271	.199	.263	.258
23	.321	.284	-.294	**.684**	-.228	.324	-.378	.271	.310	.339
37	.161	.134	-.184	**.531**	-.133	.234	-.161	.177	.167	.224
40	.338	.223	-.315	**.672**	-.267	.354	-.327	.310	.330	.376
77	.270	.152	-.282	**.781**	-.156	.297	-.247	.272	.276	.249
48	.273	.324	-.410	.314	**-.499**	.359	-.310	.291	.319	.394
50	.413	.430	-.417	.326	**-.707**	.365	-.357	.287	.380	.299

56	.425	.457	-.543	.411	**-.719**	.428	-.475	.357	.500	.406
58	.343	.305	-.468	.342	**-.703**	.365	-.341	.277	.479	.381
43	.251	.241	-.328	.340	-.206	**.752**	-.277	.283	.300	.311
57	.169	.165	-.207	.271	-.207	**.424**	-.162	.217	.122	.269
63	.204	.271	-.333	.278	-.211	**.823**	-.246	.270	.226	.289
15	.259	.392	-.366	.359	-.261	.328	**-.846**	.265	.341	.377
16	.214	.427	-.324	.336	-.239	.304	**-.859**	.243	.307	.314
35	.422	.196	-.362	.254	-.211	.302	-.231	**.559**	.302	.255
36	.546	.219	-.443	.313	-.348	.326	-.280	**.595**	.336	.304
72	.604	.194	-.470	.385	-.306	.367	-.301	**.694**	.286	.300
74	.363	.155	-.363	.251	-.174	.275	-.188	**.542**	.237	.236
79	.580	.215	-.459	.362	-.346	.350	-.270	**.741**	.342	.264
81	.380	.224	-.353	.251	-.187	.294	-.233	**.660**	.249	.216
86	.292	.075	-.271	.232	-.080	.210	-.185	**.489**	.248	.194
87	.620	.152	-.484	.430	-.227	.347	-.299	**.725**	.383	.295
88	.532	.158	-.469	.343	-.219	.328	-.264	**.689**	.344	.255

14	.453	.253	-.513	.413	-.441	.304	-.424	.303	**.727**	.359
34	.428	.277	-.513	.391	-.360	.336	-.380	.358	**.792**	.365
44	.332	.238	-.328	.340	-.231	.339	-.280	.352	**.494**	.318
64	.331	.174	-.463	.343	-.289	.328	-.305	.345	**.612**	.327
66	.354	.291	-.388	.368	-.273	.369	-.326	.405	**.590**	.329
69	.515	.250	-.672	.456	-.497	.375	-.401	.494	**.706**	.362
2	.129	.174	-.238	.259	-.091	.237	-.248	.162	.250	**.484**
31	.201	.197	-.261	.324	-.213	.288	-.281	.210	.228	**.744**
45	.067	.163	-.176	.158	-.147	.218	-.139	.131	.160	**.457**
53	.240	.207	-.337	.338	-.328	.289	-.330	.265	.235	**.602**

Presented in Table 4 are the correlations between the factors. Other than in the case of Low mood (F1) and Stress/anxiety (F8) which had an unsurprising strong correlation, correlation among the other factors was weak to moderate.

Table 4.

Factor correlation matrix

Factor	1	2	3	4	5	6	7	8	9	10
1	-									
2	.159	-								
3	-.461	-.291	-							
4	.369	.224	-.372	-						
5	-.303	-.337	.384	-.250	-					
6	.291	.319	-.404	.390	-.283	-				
7	-.267	-.454	-.369	.377	-.264	.315	-			
8	.592	.185	-.472	.327	-.221	.374	.259	-		
9	.434	.242	-.533	.410	-.345	.337	.422	.364	-	
10	.215	.274	-.378	.395	-.285	.402	.367	.287	.360	-

For the purposes of the current study, three of the 10 factors were excluded from analysis. Neck/shoulder pain (F7) was excluded due to being made up of only two items (Kline, 2005). The urination (F6) and immunity (F10) factors were excluded due to achieving Cronbach's alpha scores below .70, which is typically recognised as being the lower limit of acceptability (Nunnally, 1978). Factor analyses were repeated following the deletion of each the neck/shoulder pain, urination, and immunity factors, demonstrating no changes in the composition of any other factor.

Table 5.

Items included within each factor and omitted items

Factor	Factor structure	Comments
Depressive (*n*=6)		
	3. Repeated unpleasant thoughts 20. Crying easily over your problems 28. Feeling that your problems are disrupting your life 30. Feeling blue as a result of your problems 32. Feeling no interest in things 54. Feelings of hopelessness about the future	
Pain (*n*=6)		
	18. Arthritis 22. Leg pain or tenderness 24. Stiff or painful joints first thing in the morning 25. Joints that hurt when you move 27. Pain or tenderness in your lower back 42. Muscle soreness or stiffness	
Neurocognition (*n*=8)		
	9. Trouble remembering things 38. Having to do things slowly to ensure	

	they are done correctly 46. Difficulty in making decisions 51. Mind going blank 55. Trouble concentrating 62. Forgetfulness 70. Difficulty using words or language 78. Mental confusion or losing your train of thought	
Gastrointestinal (*n*=5)		
	17. Allergies, intolerance or reactivity to food 23. Abdominal pain or tenderness 37. Unexplained diarrhoea 40. Nausea or upset stomach 77. Symptoms of irritable bowel	
Exertion (*n*=4)		
	48. Breathlessness or chest pain upon exertion 50. Avoiding certain activities due to physical problems 56. Muscle weakness or feeling of weakness in the body 58. Unusual post exertion/exercise fatigue	

Urination (*n*=3)		Excluded due to Cronbach's alpha of less than .70
	43. Frequent Urination 57. Burning or uncomfortable urination 63. Urgent urination	
Neck/Shoulder pain (*n*=2)		Excluded due to insufficient number of items
	15. Neck pain or tenderness 16. Shoulder pain or tenderness	
Stress/Anxiety (*n*=9)		
	35. Stress from financial problems 36. Feelings that others are unsympathetic towards your problems 72. Spells of panic related to your problems 74. Frequently getting into arguments 79. Stressful events in your life related to your problems 81. Stress over family problems 86. Stress from work problems 87. Feeling anxious 88. Feelings of guilt	
Sleep/Fatigue (*n*=6)		
	14. Feeling low in energy or fatigued 34. Unrefreshed or prolonged sleep	

	44. Trouble falling asleep 64. Trouble waking up in the morning 66. Restless or disturbed sleep 69. Feelings of mental tiredness or fatigue	
Immunity (*n*=4)		Excluded due to Cronbach's alpha of less than .70
	2. Sinusitis or nasal congestion 31. Sore throat 45. Persistent cough 53. Sore or swollen lymph glands in the neck	
Omitted items (*n*=46)		
	47. Ovulation or menstruation pain	Omitted prior to analysis (sex specific)
	59. Sore or swollen lymph glands	Omitted prior to analysis (similar to item 53 and 59)
	60. Orchialgia or testicular pain	Omitted prior to analysis (sex specific)
	67. Vaginal irritation or discomfort	Omitted prior to analysis (sex specific)
	1. Headaches 4. Faintness or dizziness 5. Loss of libido or sexual interest	

	6. Night sweats, unusual sweating while asleep 7. Migraine headaches 8. Unusual muscle twitches 10. Frequent muscle cramps 11. Grinding or clenching teeth 12. Chest or heart pain 13. Face pain or tenderness 19. Poor appetite 21. Arm pain or tenderness 26. Locking or clicking or jaw 29. Tinnitus of noise in the ear 33. Photophobia or dislike of strong light 39. Heart pounding 41. Constipation 49. Hot and cold spells or recurrent feverishness 52. Loss of feeling, tingling, or numbness of the skin 61. Reactivity to smells or chemicals 65. Sore or swollen lymph glands in the groin 68. Hypersensitive skin 71. Trouble focusing your eyes 73. Sciatica or numbness/tingling down	

	the back of the leg 75. Cold hands 76. Recurrent mouth ulcers 80. Dermatitis	

References

Costello, A. B., & Osborne, J. W. (2005). Best practices in exploratory factor analysis: four recommendations for getting the most from your analysis. *Practical Assessment, Research, & Evaluation,* 10, 1-9.

Fabrigar, L. R., Wegener, D. T., MacCallum, R. C., & Strahan, E. J. (1999). Evaluating the use of exploratory factor analysis n psychological research. *Psychological Methods,* 4(3), 272-299. http://dx.doi.org/10.1037/1082-988X.4.3.272

Kline, R. B. (2005). *Methodology in the social sciences.Principles and practice of structural equation modeling (2nd ed.).* Guilford Press.

Nunnally, J. C. (1978). *Psychometric theory* (2nd ed.). New York: McGraw-Hill.

Pallant, J. (2010). *SPSS survival manual: a step by step guide to data analysis using SPSS* (6th ed.). Maidenhead, UK: Open University Press/McGraw-Hill.

Chapter 6: Associations Between Self-Reported Psychological Symptom Severity and Gut Microbiota: Further Support for the Microgenderome

Given the results of Paper 2 which suggest that *Blastocystis* and *D. fragilis* do not effect psychological symptom expression, those who carried these intestinal protozoa were not excluded from the analyses conducted in this chapter (Paper 3). Paper 3 explores the associations between bacterial and fungal microbiota species and psychological symptom expression.

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Associations between self-reported psychological symptom severity and gut microbiota: further support for the microgenderome

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Abstract

Background: Research into the brain-gut-microbiota axis (BGMA) continues to reveal associations between gut microbiota (GM) and psychological symptom expression, inspiring new ways of conceptualising psychological disorders. However, before GM modulation can be touted as a possible auxiliary treatment option, more research is needed as inconsistencies in previous findings regarding these associations are prevalent. Additionally, the concept of the microgenderome, which proposes that GM may interact with sex hormones, has received limited attention in studies using human samples to date. However, such research has demonstrated sex specific associations between GM and psychological symptom expression.

Method: This cross-sectional retrospective study explores associations between GM species (identified through faecal microbial analysis) and symptom severity across four psychological domains (Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue) for males ($N = 1143$) and females ($N = 3467$) separately.

Results: GM species from several genera including *Bifidobacterium*, *Clostridium*, *Enterococcus*, and *Leuconostoc* were found to be differentially associated with psychological symptom severity for males and females. As such, the findings of the current study provide support for the concept of the microgenderome.

Conclusion: While further research is needed before their implementation in psychological treatment plans, the current findings suggest that modulation of GM at the species level may hold promise as auxiliary diagnostic or treatment options. These findings may give further insight into a client's presenting problem from a more holistic, multidisciplinary perspective. The clear sex divergence in associations between GM and symptoms give insight into sex discrepancies in susceptibility to psychological disorders.

Keywords: Gut microbiota, Psychological symptoms, Microgenderome, Brain-gut-microbiota axis, Psychology

Background

The proliferation of research into the brain-gut-microbiota axis (BGMA) has continued to demonstrate associations between gut microbiota (GM) and psychological symptom expression [1–8]. It is well established that the trillions of microorganisms in the gut that comprise the GM have co-evolved to share a symbiotic relationship with their human host [9, 10]. The GM is a diverse and

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highly complex ecosystem comprising of bacteria, fungi, protozoa, viruses, and archaea [11]. GM are believed to influence host health (and alternatively symptom expression) though neuronal, immune, endocrine, and metabolic pathways [12–14]. However, the precise combination of microorganisms that constitutes a 'healthy' GM has not been established due to immense intra- and inter-person variability. While more than 2000 microbial species have been identified [10], the number of species within an individual's GM has been suggested to range between approximately 100–500 [15, 16]. As such, the possible combinations that may exist within and between individuals are exponential.

There is an abundance of research demonstrating the role of GM among psychosocial behaviours including mood, cognition, stress, and sleep in preclinical murine models, albeit studies involving human participants are to date limited [17]. Research involving human participants provides mounting evidence that changes in GM composition, or the abundance of specific microbes, may be associated with psychological symptom expression and disorders. While recent systematic reviews provide evidence of such associations, they also highlight heterogenous and at times contradictory findings, with few taxa being associated with symptom expression across multiple studies [18–22]. One reason for this may be that the majority of studies investigating associations between GM and psychological symptoms investigate the GM at either the genus level or higher. While this provides valuable information, the nuances of more specific species level information is missed. Gaining a more precise understanding of associations between GM and symptom expression at a species level is imperative because the heterogeneity of species within a single genus makes it difficult to prescribe health benefits or detriments at the genus taxonomic rank. Another possible reason for these discrepancies in findings is that there are a number of different techniques commonly used to analyse the presence and abundance of GM [23, 24]. These include culture-based methods, which involve growing selected bacteria and estimating the number of viable (live) cells in a sample, versus DNA based techniques such as 16 rRNA sequencing, and shotgun sequencing which include both live and dead cells. The strengths and weaknesses of these different techniques are discussed elsewhere [25].

Microgenderome: sex differences in microbe-host relationships

Research supports a complex multidirectional relationship between sex hormones, GM, and immunity which all exist within the broader BGMA communication network [26–28]. Specifically, growing evidence supports the concept of the microgenderome which implies that sex hormones play a role in modulating GM, therefore resulting in sex-specific host-microbiota interactions [29–31]. While sex differences in microbiota composition have mostly been demonstrated in pre-clinical studies, human studies have provided support for the microgenderome [31–38]. Compositional sex differences have been noted in various genera such as *Bacteroides, Bilophila, Escherichia, and Veillonella* [34, 37]. Meanwhile, Wallis et al. [31] found GM composition to be similar between the sexes, however, *Clostridium*, *Lactobacillus*, and *Streptococcus*, were associated with physical and psychological symptoms in a sex divergent manner. Associations between psychological symptoms and some species of fungi have also demonstrated sex divergence. For example, *Candida albicans* has been found to be associated with cognitive deficits in schizophrenia and bipolar disorder in a sex specific manner [38]. These sex-specific interactions may offer some insight into differing prevalence rates between males and females for various disorders which have been associated with certain gut microbiota profiles such as autism, IBS, anxiety, and depression [39].

The current study

The current study extends upon work conducted by Wallis et al. [31] who investigated sex specific relationships between microbial genera and symptom expression in a sample of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) sufferers. While Wallis et al. [31] specifically investigated bacterial genera, they suggest the need to investigate the host relationship with microbes at the species level. The current study explored the relationship between an array of microbial species (including bacteria and fungi) in a large, clinically diverse sample.

The aim of the current study was to investigate the relationship between GM at the species level and symptom severity across four psychological symptom domains (Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue) between males and females. It was hypothesised that the pattern of relationships between GM species and psychological symptom expression would differ between males and females.

Methods

Participants

The current study consisted of a retrospectively collected sample of 4610 (1143 males and 3467 females) clinically diverse adult patients ranging in age from 18 to 87 years ($M = 43.09$, $SD = 13.48$). While females clearly outnumbered males, there were sufficiently robust numbers in each sample. Patients were referred to Bioscreen, a Melbourne based laboratory specialising in faecal microbial analysis (FMA), between February 2013 and June 2015.

Stool samples were submitted for analysis as part of the investigatory process for intestinal dysbiosis however, the specific diagnostic status of patients were not identified. As such, this is a cross-sectional study of a broad range of potentially clinical and non-clinical presentations. Ethics approval for the current study was obtained from the Victoria University Human Research Ethics Committee (HRE16–071). The Bioscreen Patient Questionnaire included a statement that patients' data would be used for research purposes, however, patients had the option to 'opt out' (by ticking a box), in which case, consent was not provided, and their data was not included in any analyses.

Sample collection and microbial identification

Stool samples were collected by patients according to directions provided in a Bioscreen FMA kit which was posted to their home. The FMA kit contained a faecal collection tub (anerobic pouch system) with a perforated lid to aid anaerobiasis, which was achieved by activating an Anaero Gen Compact (Oxid, Thermo Fisher, Australia), a zip lock bag, and three icepacks. Samples were transported to the laboratory in polystyrene boxes with three frozen icepacks to maintain a temperature below 12 °C. Stool samples were transported via Express post meaning that sample would arrive at Bioscreen for analysis within 48 h of collection. Faecal samples that were incorrectly collected or transported, or those which were subjected to inaccurate anaerobiosis or refrigeration procedures, were rejected according to Bioscreen's laboratory protocol.

Sample analysis

Faecal microbial analysis (FMA) The FMA process described here was identical to that used in Coulson et al. [40] and Wallis et al. [31] Given that the current study extends on work previously conducted within the same research team at Victoria University by Wallis et al. [31], the procedures outlined below are exactly the same. Given the specificity of FMA, the following information has been sourced from Coulson et al. [40] and Wallis et al. [31]

Once removed from the aerobic collection tub, samples were processed within 10 to 15 min. Between 0.5 and 1.0 g of stool was transferred to 10 mL of 1% glucose-saline buffer. Dilution factor was determined by the difference in the weight of the glucose-saline buffer with and without the sample. One hundred and one thousand-fold dilutions (beginning from 10^{-1} to 10^{-7} of homogenised faecal samples were prepared. Dilutions (10 and/or 1 µL amounts) were transferred onto previously dried Columbia horse blood agar (Oxid), chromogenic medium (Oxid), colistin and nalidixic acid blood selective agar (Oxid), and chloramphenicol-gentamicin selective Sabouraud agar for aerobic incubation. Aerobic media were incubated at 35 °C for 48 h. Columbia horse blood haemin agar and Raka Ray medium were used for anaerobic incubation in anaerobic jars (Oxid) for a duration of 4 days. A stereomicroscope was used to examine aerobic and anaerobic culture plates for a minimum of 20 min per plate before bacterial identification. Each colony from each medium was microscopically examined and the colony/viable count were quantified. To assess purity prior to identification, similar morphotypes were sub-cultured onto horse blood agar.

Following these purity checks (overnight), index bacterial colonies were transferred to a target polished steel plate (MSP 96, Bruker Daltonics Inc.) for drying under exhaust ventilation in a Class II Biohazard Hood (Gelman Sciences Australia) at room temperature. Air-dried samples were then subject to protein extraction with 1 µL 70% formic acid (Sigma). After again being allowed to dry under exhaust ventilation, samples were overlain with 1 µL of matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid [HCAA] in a mixture of 47.5% ultra-pure water, 2.5% trifluoracetic acid, and 50% acetonitrile). Once dried, samples were then analysed using a Microflex matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (Bruker Daltonik GmbG, Leipzig, Germany) equipped with a 60 Hz nitrogen laser. Spectra were recorded in the positive linear mode for the mass range of 2000 to 20,000 Da at maximum laser frequency. Raw spectra were analysed using the default settings of the MALDI Biotyper 3.0 software package (Daltonik GmbH, Bremen, Germany) which can detect approximately 5000 species. The most prevalent microorganisms were quantified as colony forming units (CFU)/g.

Bioscreen patient questionnaire (BPQ) The BPQ is an 88-item questionnaire developed by Bioscreen administered to patients as part of Bioscreen's standard procedure. Items on the BPQ are similar in nature to other symptom checklists which relate to diverse symptomatology and patients are asked to report the frequency (over the past 12 months) and severity (over the past 7 days) of their symptoms on a five-point Likert scale ranging from zero to four, with higher scores indicating higher ratings of frequency/severity. For the purposes of the current study, only symptom severity over the past 7 days was assessed, given the underlying assumption of a temporal relationship between the presence of specific GM and symptom expression. The BPQ has been used

in previous studies such as Wallis et al. [31] who used clinically derived factors, and Ganci et al. [41] who subjected the severity items of the BPQ to an exploratory factor analysis. While 10 factors were derived, only the four psychological symptom domains were included in the current study which include Depressive symptoms (6 items; Cronbach's $\alpha = 0.894$), Neurocognitive symptoms (8 items; Cronbach's $\alpha = 0.938$), Stress and Anxiety symptoms (9 items; Cronbach's $\alpha = 0.874$), and Sleep and Fatigue symptoms (6 items; Cronbach's $\alpha = 0.853$). Possible score ranges for each symptom domain can be found in Table 1, with higher scores indicating the endorsement of greater symptom severity.

Data handling and statistical design Both aerobic and anaerobic bacterial species and fungi were analysed in the current study. Data regarding intestinal protozoa were investigated in a previous study [41] and are not included here. Viruses, which are also part of the gut microbiota, were not analysed as they were not included in the retrospectively collected data.

A total bacterial count CFU/g was calculated by adding the viable CFU/g counts across all bacterial species, with total aerobe CFU/g and total anaerobe CFU/g counts also being calculated by adding only aerobic or anaerobic bacteria respectively. The total fungal CFU/g count was calculated in the same way, by adding the CFU/g counts for each fungal species.

Four hundred and ninety-five bacterial and fungal species were identified in the overall sample of Bioscreen patients over the two and a half years of testing between 2013 and 2015. Of the 495 bacterial and fungal species identified, 132 species were found to have a viable count in only a single patient, and a further 188 species were identified in between only two and fourteen patients ($< 0.5\%$ of the patient sample). Therefore, a total of 175 species (species that had a viable count in ≥ 15 patients) were analysed to determine if there were significant differences between the viable CFU/g count in males and females. Pairwise deletion was used to deal with missing data. Given that many variables had a large proportion of missing data (ranging from 32 to 99.98% missing), data imputation methods would not have been appropriate [42]. Furthermore, Kaul et al. [43] refers to missing data in microbiome research as "structural zeros" which are due to an individual's underlying biology. For example, if an individual harbours a combination of between 100 and 500 species of GM [15, 16], but over 2000 species have been identified [10], it is highly unlikely that any two individuals would have the same combination of microorganisms, resulting in high levels of missing data as observed in the current study. Therefore, Kaul et al. [43] argue that the use of typical imputation methods would be erroneous. As such, the sample size for comparisons of CFU/g between males and females, and associations between microbial species and psychological symptom severity varied by species. While many of the microbial species analysed were detected in a substantive number of patients, several other microbes were detected in very few patients (see Table 2 and Figs. 1, 2, 3, & 4). The retrospective nature of the current study precluded the collection of further data. For example, age could not be considered due to missingness in the data which would mean that sample sizes would be even further reduced. Moreover, the retrospective dataset did not include information regarding an individual's life stage (i.e. menopause).

As the assumptions of parametric statistics were violated for CFU/g counts, namely normality and the presence of outliers, sex differences in microbial counts were analysed using a series of Mann-Whitney *U* tests. While nonparametric tests have less statistical power, it is argued that when the assumption of normality is violated or when outliers are present, nonparametric tests are clearly the correct choice [44, 45], especially in small samples [46, 47]. While the overall sample size of the current study was large, sub-samples of individuals with specific microorganisms were often small within each sex. While there is no consensus over what is considered to be a minimum sample size, particularly for nonparametric tests, it has been suggested that a Mann-Whitney *U* test requires a minimum of 4 participants per group

Table 1 Descriptive information for males and females relating to differences in symptom severity

Symptom domain (possible range)	Males			Females		
	n	*M*	*SD*	*n*	*M*	*SD*
Depressive (0–24)	1071	7.469	6.266	3265	9.055	6.702
Neurocognitive (0–32)	1074	10.077	8.705	3291	12.336	8.922
Stress and Anxiety (0–36)	1059	8.062	7.558	3217	10.094	8.144
Sleep and Fatigue (0–24)	1085	10.129	6.440	3297	12.351	6.320

Table 2 Statistically significant results of Mann-Whitney U test assessing difference in the viable CFU/g count of species between males and females

Species	Male		Female		*U*	*z*	*p*	*r*
	n	Median (*Md*)	*n*	Median (*Md*)				
Alistipes finegoldii	70	$7.215*10^9$	274	$3.835*10^9$	7489	−2.829	.005	.153
Bacteroides uniformis	567	$7.400*10^9$	1841	$6.090*10^9$	481,924	−2.763	.006	.056
Bacteroides vulgatus	745	$8.000*10^9$	2287	$6.070*10^9$	775,833.5	−3.666	<.001	.067
Bifidobacterium animalis	169	$1.350*10^8$	665	$9.430*10^7$	48,876.5	−2.616	.009	.091
Collinsella aerofaciens	631	$7.020*10^9$	1710	$5.315*10^9$	486,257	−3.669	<.001	.076
Candida albicans	314	2250	914	1380	130,007	−2.489	.013	.071
Lactobacillus gasseri	103	$2.130*10^6$	272	$7.245*10^5$	11,220.5	−2.975	.003	.154
Lactobacillius rhamnosus	83	$2.070*10^6$	311	$1.050*10^6$	11,019.5	−2.047	.041	.103
Lactobacillus salivarius	37	$7.490*10^6$	134	$1.860*10^6$	1932	−2.052	.040	.157
Parabacteroides goldsteinii	13	$4.800*10^8$	58	$1.860*10^9$	237	−2.082	.037	.247
Ruminococcus gnavus	30	$3.655*10^9$	124	$1.845*10^9$	1397	−2.112	.035	.170
Streptococcus salivarius	533	$5.870*10^6$	1337	$3.000*10^6$	305,494	−4.821	<.001	.111
Streptococcus parasanguinis	523	$3.060*10^6$	1404	$1.980*10^6$	322,444.5	−4.116	<.001	.094
Streptococcus gordonii	64	$2.640*10^6$	139	$1.010*10^6$	3320	−2.901	.004	.204

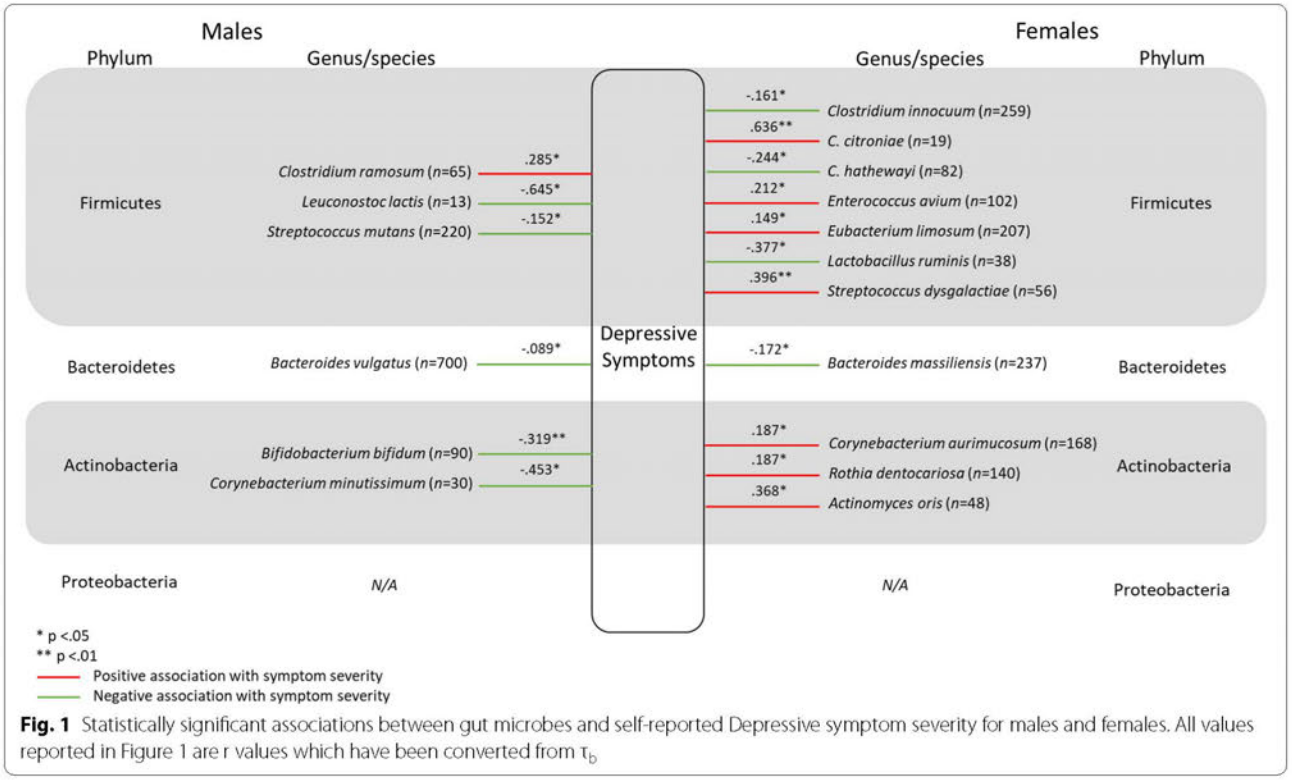

Fig. 1 Statistically significant associations between gut microbes and self-reported Depressive symptom severity for males and females. All values reported in Figure 1 are r values which have been converted from τ_b

($N=8$) before having the possibility of rejecting the null hypothesis [48]. However, given that there is consensus that smaller samples provide less valid findings [49–51], a higher minimum number of cases ($n \geq 10$) was used as the benchmark for consideration in the current study. The authors acknowledge the limitations of the current data set and suggest that the results of the current study be interpreted with caution, and should be considered as exploratory. As such, no adjustments were made to the alpha levels. While the issue of alpha value adjustment

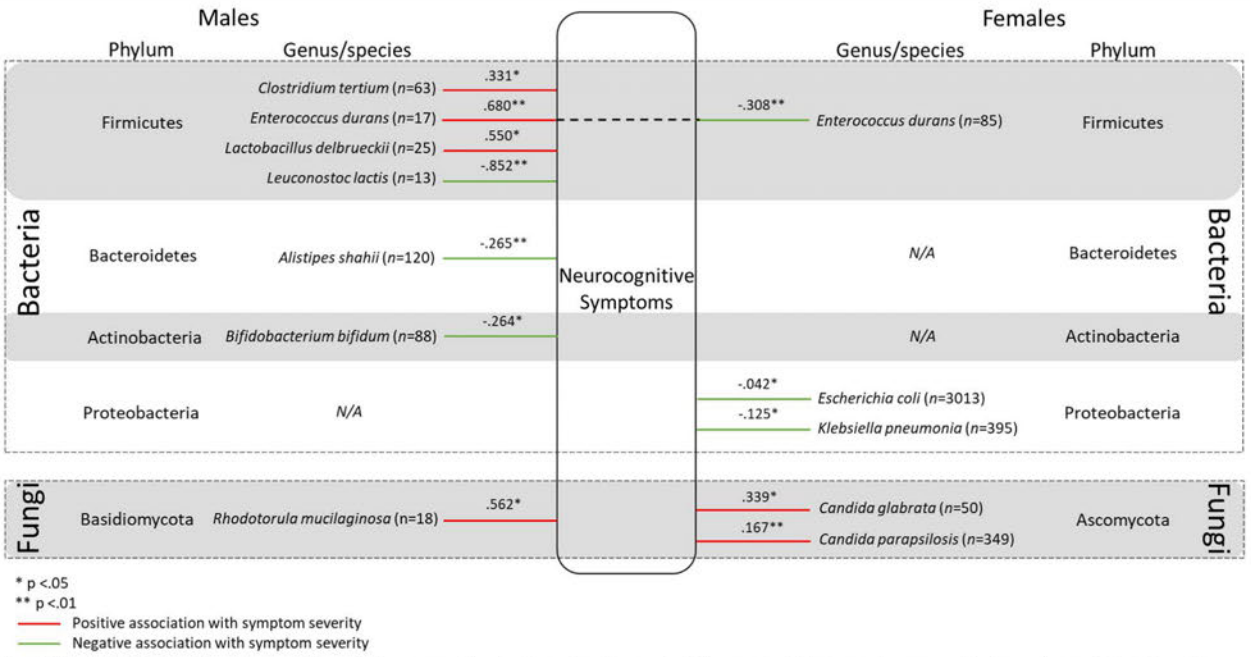

Fig. 2 Statistically significant associations between gut bacteria and self-reported Neurocognitive symptom severity for males and females. All values reported in Figure 2 are r values which have been converted from τ_b

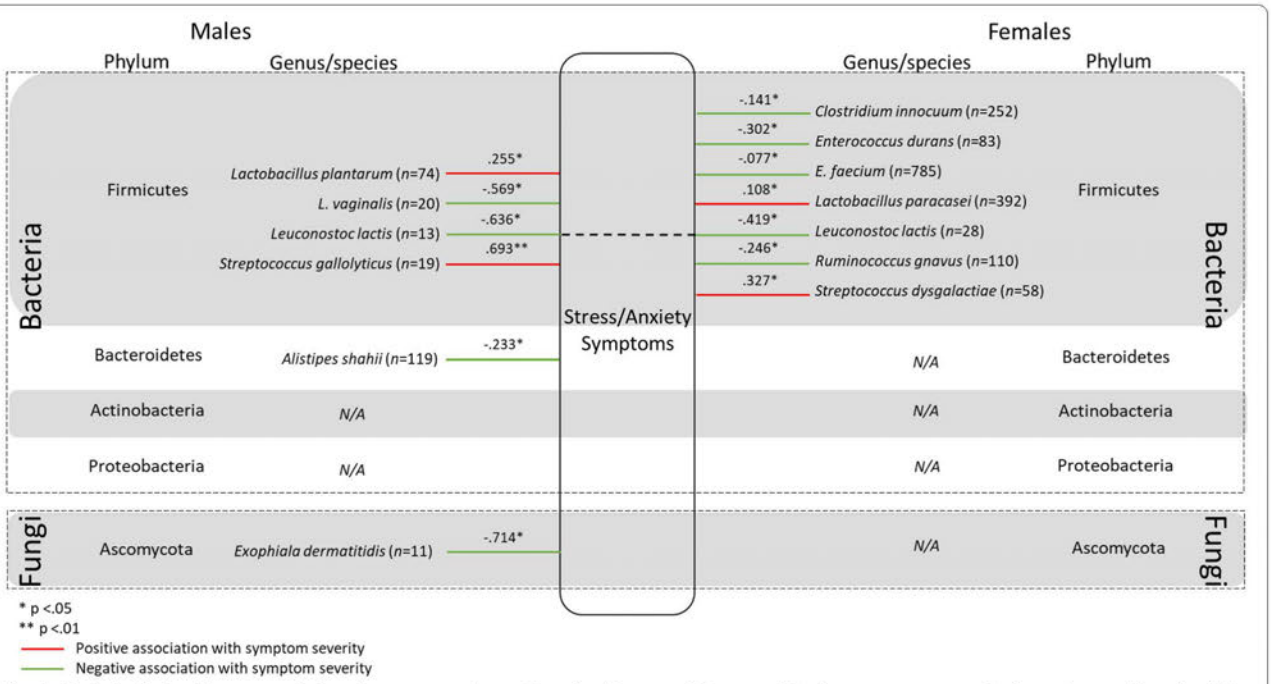

Fig. 3 Statistically significant associations between gut bacteria and self-reported Stress and Anxiety symptom severity for males and females. All values reported in Figure 3 are r values which have been converted from τ_b

is somewhat contentious, Rubin [52] argues that it is not necessary for exploratory analyses. It is also argued that alpha adjustments are only necessary when multiple tests are used to test the same hypothesis within the exact same population [52], which was not the case in the current study.

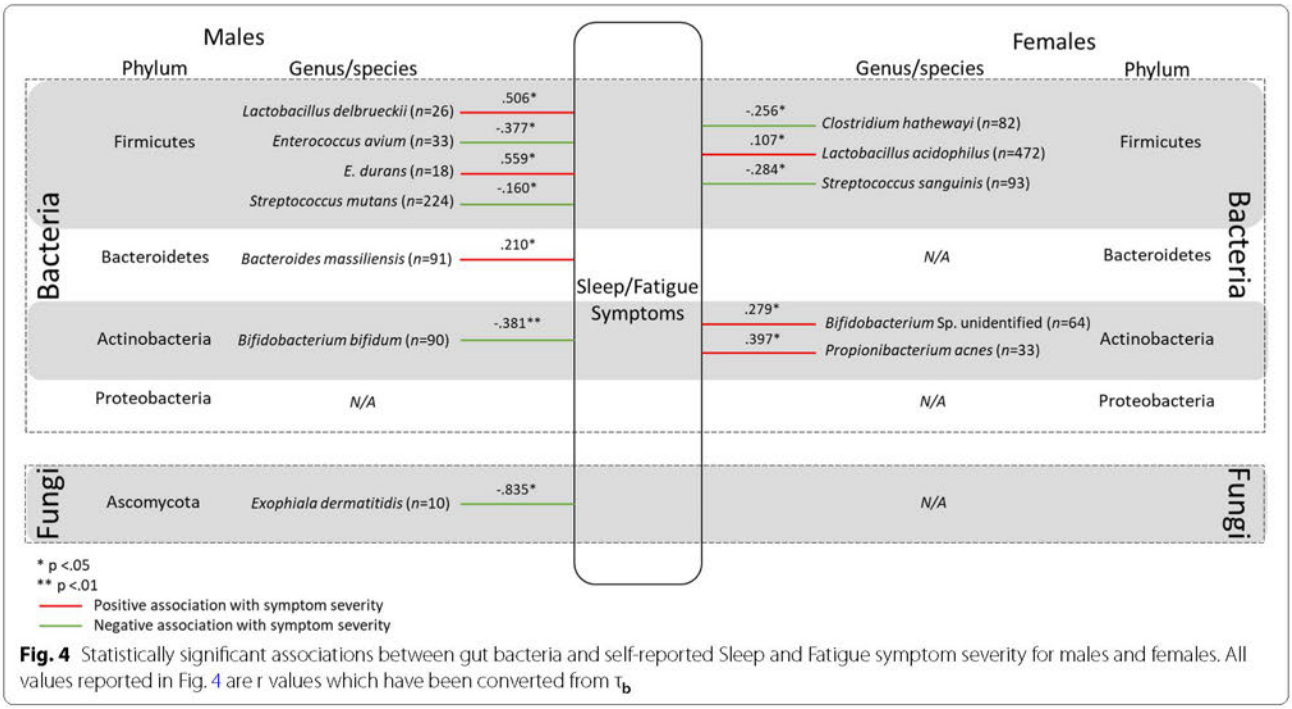

Fig. 4 Statistically significant associations between gut bacteria and self-reported Sleep and Fatigue symptom severity for males and females. All values reported in Fig. 4 are r values which have been converted from τ_b

Reported z scores from the Mann-Whitney U analyses were used to calculate an approximate value of r as a measure of effect size using the formula (1):

$$r = \frac{z}{\sqrt{N}} \tag{1}$$

Further analyses using a series of Kendall's Tau-b (τ_b) correlations were performed to determine the monotonic relationship between gut microbiota and psychological symptom severity across the domains of depressive, neurocognitive, stress and anxiety, and sleep and fatigue symptoms. Some authors have suggested that Kendall's τ_b may draw more accurate generalisations compared to Spearmans rho [53]. Correlations were interpreted only in cases where the sample size was at least 10 in both males and females (given that correlations between GM and psychological symptom severity were run separately for each sex) as it has been suggested that Kendall's τ_b performs reasonably well with small samples ($n \geq 10$), but also under a variety of other conditions and sample sizes [54]. As such, 152 GM species were analysed to determine correlations between GM and psychological symptom severity. Long and Cliff [54] go so far as to suggest that Kendall's τ_b should be considered a worthy measure of correlation in its own right, and not only as an alternative to Pearson's correlation coefficient, also making it a suitable choice for larger samples.

In order to aid in the interpretation of the strength of the relationships identified using Kendall's τ_b, τ_b values were converted to r using formula (2) [55].

$$r = \sin(.5\ \pi\ \tau) \tag{2}$$

All values presented in Figures throughout the results section are r values which were converted from Kendall's τ_b values using (2). See Table S1 for the original Kendall's τ_b values, along with the associated r, r^2, n, Fisher's z transformation (z_r), and p values for each species associated with the four psychological symptom factors for males and females.

In cases where the same species of gut microorganism was found to be associated with a psychological symptom factor in both males and females, the strength of the relationship between that microbe and symptom severity for each sex was compared using Fisher's z-transformation (Z_r) of r using formula (3).

$$z_r = \frac{1}{2}\, ln[(1+r)/(1-r)] \tag{3}$$

Once the r for males and females were converted into z scores, formula (4) was used to determine if there was a significant difference in the strength of the associations.

$$z_{obs} = \frac{z_1 - z_2}{\sqrt{\frac{1}{N_1 - 3} + \frac{1}{N_2 - 3}}} \quad (4)$$

Results

Sex differences in self-reported symptom severity

Presented in Table 1, are the mean and standard deviation symptom severity scores for the four psychological domains measured. As can be seen, females self-reported greater symptom severity across all four psychological symptom domains. A series of independent samples *t*-tests were run to determine whether these differences were statistically significant.

It was found that females' self-reported symptom severity was significantly higher compared to males for Depressive symptoms, t (1934.423) = −7.062, $p < .001$, $d = .244$, Neurocognitive symptoms, t (4363) = −7.247, $p < .001$, $d = .256$, Stress and Anxiety symptoms, t (1928.529) = −7.440, $p < .001$, $d = .259$, and Sleep and Fatigue symptoms, t (4380) = −9.996, $p < .001$, $d = .348$. According to suggested benchmarks [56], the effect size for each symptom domain was small, suggesting that sex alone only accounts for a small proportion of variance in symptom severity.

Sex differences in bacterial composition

Mann-Whitney *U* tests showed that at the most global level, statistically significant sex differences were found between males ($n = 1143$) and females ($n = 3467$) in regards to viable total bacterial count ($Mdn_{males} = 2.98 \times 10^{10}$, $Mdn_{females} = 2.64 \times 10^{10}$, $U = 1{,}853{,}660$, $z = -3.259$, $p = .001$, $r = .048$), total aerobe count ($Mdn_{males} = 5.77 \times 10^{7}$, $Mdn_{females} = 4.28 \times 10^{7}$, U = 1,848,775.5, $z = -3.398$, $p = .001$, $r = .050$), and total anaerobe count ($Mdn_{males} = 2.96 \times 10^{10}$, $Mdn_{females} = 2.60 \times 10^{10}$, $U = 1{,}853{,}741$, $z = -3.257$, $p = .001$, $r = .048$), with males demonstrating higher median CFU/g counts in all three instances. Additionally, the total fungal CFU/g count was also found to be significantly higher in males ($n = 1074$) compared to females ($n = 3281$; $Mdn_{males} = 186.500$, $Mdn_{females} = 110$, U = 1,687,589.500, $z = -2.189$, $p = .029$, $r = .033$). Using McGrath and Meyer's [57] guidelines for the interpretation of *r* (based on Cohen [56]), the effect sizes of these differences were all very small, suggesting that the statistical significance was likely a statistical artifact due to the large sample.

Of the 175 species analysed using Mann-Whitney *U* tests, the viable CFU/g count of 14 species (13 bacterial and one fungal) were found to be significantly different in abundance between males and females. The species identified as exhibiting sex differences in viable CFU/g counts are displayed in Table 2.

As can be seen in Table 2, for microbes which showed a significant difference in abundance between males and females, males had a higher count in all but one GM species, *Parabacteroides goldsteinii*.

While statistically significant, the effect sizes of the differences in CFU/g between males and females were low or negligible, as can be seen in Table 2. This suggests the statistical significance is either due to statistical artifact, or that sex alone only accounts for a very small portion of variance in microbial CFU/g counts between males and females. Considering that only 14 out of 175 species of gut microbes analysed demonstrated a significantly different abundance between males and females, and the low to negligible effect sizes, the overall snapshot provided by these results suggests a predominantly similar microbial composition between the sexes.

Sex dependent relationships between gut microbes and psychological symptomatology

While composition was found to be mostly similar between males and females, the results showed that associations between gut microbes and psychological symptom severity varied in a sex dependent manner across all four psychological symptom factors. Of the 152 GM species analysed, 39 different microorganisms (35 bacterial and four fungal) were found to be associated with at least one of the four psychological symptom domains.

Positive associations between a microbial species and psychological symptoms indicate that an increase in the rank of viable CFU/g count was monotonically associated with an increase in the rank of symptom severity. That is, a positive correlation indicates that the species is associated with more severe symptoms. Conversely, a negative association indicates that an increase in the rank of viable CFU/g count was monotonically associated with a decrease in the rank of symptom severity. That is, a negative correlation indicates that the species is associated with less severe symptoms.

Depressive symptoms

Relationships between GM and Depressive symptom severity differed in a sex dependent manner. There were more bacterial species associated with Depressive symptom severity in females compared to males, particularly from within the Firmicutes phylum. *Clostridium ramosum* was the only microbe found to be positively associated with Depressive symptom severity in males and *Leuconostoc lactis* demonstrated the strongest negative relationship with symptom severity. For females, *C. citroniae* showed the strongest positive association with symptom severity, while *Lactobacillus ruminis* demonstrated the strongest negative relationship with symptom severity. See Fig. 1 for all statistically significant

associations between GM and Depressive symptom severity in males and females. Statistically non-significant correlations are not included in the figure.

Neurocognitive symptoms

The associations between GM and Neurocognitive symptom severity again showed a different pattern between males and females. *L. lactis* once again showed the strongest negative association with symptom severity in males. On the other hand, *Enteroccocus durans* demonstrated the strongest positive association with symptom severity in males. Conversely, in females, *E. durans* demonstrated a weak negative association with symptom severity. Not only was the direction of the association contrasting between males and females for this species, but there was also a significant difference in the strength of the association between sexes ($z_{obs} = 7.479$, exceeding the critical value of 1.96). See Fig. 2 for all statistically significant associations between gut bacteria and neurocognitive symptoms in males and females. Statistically non-significant correlations are not included in the figure.

Stress and anxiety symptoms

The overall pattern of associations between GM and Stress and Anxiety symptom severity was largely distinct between males and females. *L. lactis* again showed a strong negative association with symptom severity in males. Demonstrating sex consistency, *L. lactis* also showed a moderate negative association with symptom severity in females. The difference in the strength of the associations between males and females for *L. lactis* did not exceed the critical value of 1.96 and was therefore not statistically different ($z_{obs} = -1.634$). See Fig. 3 for all statistically significant associations between GM and Stress and Anxiety symptom severity in males and females. Statistically non-significant correlations are not included in the figure.

Sleep and fatigue

Again, the pattern of associations between GM and Sleep and Fatigue symptom severity was different between males and females. Several GM demonstrated similar associations with Sleep and Fatigue symptom severity as they did with other symptom domains including negative associations with *B. bifidum* and *Exophiala dermatitidis* and positive associations with *L. delbrueckii* and *E. durans*. See Fig. 4 for statistically significant associations between gut microbes and sleep and fatigue symptom severity for males and females. Statistically non-significant correlations are not included in the figure.

Discussion

The aim of the current study was to investigate the relationship between GM and symptom severity across four psychological domains (Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue symptoms) and to assess whether these relationships differed between males and females. The hypothesis that the pattern of relationships between GM and psychological symptom severity would vary between males and females was supported. There was clear divergence in the patterns and species of GM associated with symptom severity in males and females across all four psychological symptom domains. As such, these findings provide further support for the concept of the microgenderome in a human sample.

The current study found that females endorsed greater symptom severity across all four psychological domains which is consistent with previous research [58–62]. Barsky et al. [59] suggest that socialised gender expectations may explain differences in symptom reporting between males and females. Others have suggested that sex differences in symptom expression are based on interactions between sex hormones and serotonergic and glutamate systems [63, 64]. Sex differences in immune functioning have also been proposed to explain the increased prevalence of mood and anxiety disorders in females [65, 66]. However, the small effect sizes found in the current study suggest that sex alone explains only a small amount of variance in self-reported psychological symptom severity.

Of the 39 species found to be associated with psychological symptom severity, only *Ruminococcus gnavus* was found to have significantly different counts between males and females (as seen in Table 2). Gao et al. [33] found the genus *Ruminococcus* to vary according to gender, finding it to be more abundant in females, which has also been reflected in murine models [67]. However, the current study found *R. gnavus* to be more abundant in males. Although somewhat contradictory, these findings suggests that Ruminococci species may be particularly vulnerable to the influence of sex hormones. This discrepancy may be due to the fact that the current study investigated sex differences at the species level whereas Gao et al. [33] reported differences at the genus level. This means that perhaps other species within the *Ruminococcus* genus may collectively make the genus more abundant in females. This possibility supports the need for future research to be conducted at the more specific species level to provide a clearer and more precise indication of the associations between GM, sex hormones, and symptom expression.

All other species found to have a relationship with symptom severity in the current study showed a similar abundance in males and females, demonstrating that

sex-divergent associations between GM and symptom severity were not due to underlying differences in GM composition. This finding is similar to that of Wallis et al. [31] who also found sex specific correlations between bacterial genera and a variety of physical and psychological symptom domains despite similar bacterial composition between the sexes.

Part of the rationale for the current study was the need to explore associations between GM and psychological symptoms at the species level in contrast to the majority of previous research focusing of genus level and above. The results of the current study show that, for example within the *Clostridium* genus, certain species were positively associated with symptom severity (*C. citroniae, C. ramosum,* and *C. tertium*), while other species within this same genus were negatively associated with symptom severity (*C. innocuum* and *C. hathewayi*). These findings are consistent with previous research having shown similar disparities between *Clostridium* species [68, 69]. If measured at the genus level, these nuances would have been missed, and important associations may have been masked.

Depressive symptoms

Previous findings have established that individuals with depression have markedly different GM composition [3, 18–22, 32]. Depression is considered to be the leading cause of disability worldwide [70]. As such, research into the BGMA offers valuable information regarding possible biological contributions to the pathogenesis of the disorder [71]. Furthermore, the differing patterns of associations between GM and depressive symptoms in males and females may offer insight into sex differences in prevalence rates of the disorder.

The findings of the current study demonstrated a greater number of species were found to be associated with Depressive symptoms in females compared to males. *Clostridium ramosum* was the only bacterial species found to be positively associated with Depressive symptom severity for males, showing a weak association. On the other hand, *Leuconostoc lactis* was found to have a moderate negative association with Depressive symptom severity in males. *L. lactis* was also found to be negatively associated with both Neurocognitive and Stress and Anxiety symptom severity in males. The seemingly protective nature of *L. lactis*, at least for males, may be explained by previous studies which have found the species to have anti-inflammatory properties [72, 73], to inhibit the effects of pathogenic bacteria and fungi [74], and to be associated with improved cardiovascular health [75]. For females, *Clostridium citroniae* was positively associated with symptom severity, however, *C. innocuum* and *C. hathewayi* were negatively associated. As such, this demonstrates the need for research to be conducted at the species level to provide more detailed and precise information regarding GM and their association with symptom expression. *Lactobacillus ruminis* also demonstrated a negative association with symptom severity in females.

For males, *Bifidobacterium bifidum* was found to be negatively associated with Depressive symptom severity and was also found to be negatively associated with both Neurocognitive and Sleep and Fatigue symptom severity. These findings are in line with previous research purporting health benefits of *B. bifidum* [76–78]. The results are also in line with those of Aizawa et al. [79] who, at the genus level, found *Bifidobacterium* to be reduced in those diagnosed with depression. For females, *B. bifidum* was not found to be associated with any of the four psychological symptom domains measured, suggesting that the species' health promoting effects may be sex dependent. The current findings support the notion that species from the *Bifidobacterium* genus act in a sex dependent manner [80]. However, the sex effect was reversed in the current study as Luk et al. [80] found that, at the genus level, *Bifidobacterium* ameliorated anxiety like behaviours only in female mice. It is possible that measurement of GM at the broader genus level masks underlying sex differences in specific species within a genus, warranting further research at the species (and/or strain) level.

In females, *Corynebacterium aurimucosum, Rothia dentocariosa,* and *Actinomyces oris* (species belonging to the Actinobacteria phylum) demonstrated a positive association with symptom severity. This finding is in keeping with those of Chen et al. [32] who found a greater abundance of phylum Actinobacteria in females diagnosed with depression, but not males. Sex divergent associations were demonstrated within the *Corynebacterium* genus as *C. minutissimum* was found to be negatively associated with Depressive symptoms in males. *Corynebacterium* species have previously been demonstrated to produce serotonin in culture mediums [81] which may generally explain their association with Depressive symptoms, however it does not explain why these effects were sometimes reversed between the sexes. Although further research is necessary to clarify the role of *Corynebacterium* in serotonin production, the findings of the current study demonstrate that the role of sex hormones should be considered in any such investigations.

Demonstrating some consistency between the sexes, *Bacteroides* species (*B. vulagtus* in males and *B. massiliensis* in females) were also found to be negatively associated with Depressive symptom severity. This finding was in line with those of Jiang et al. [3] who found *Bacteroides* at the genus level to be depleted in patients with depression, however this is in contrast to the findings of

Liu et al. [82] and Rong et al. [7] These inconsistencies suggest the need for further investigation into association between *Bacteroides* species and Depressive symptoms.

Neurocognitive symptoms

While neurocognitive symptoms are not themselves a specific disorder, they occur across numerous physiological and psychological disorders [83–85]. Brain fog is a form of neurocognitive impairment which features mental confusion, impaired judgement, deficits in short term memory, and difficulty concentrating [86]. Given the associations reported in the literature between brain fog and bacterial overgrowth [86], the current investigation of potential sex differences in the pattern of associations was largely exploratory. Severance et al. [38] found *Candida albicans* IgG to be differentially associated with neurocognitive symptoms in males and females with schizophrenia and bipolar disorder. The pattern of associations between GM species and Neurocognitive symptom severity in the current study also differed between males and females. For males, *Clostridium tertium*, *Enterococcus durans*, and *Lactobacillus delbrueckii* were positively associated with symptom severity. As aforementioned, *L. lactis* was found to be negatively associated with symptom severity in males. Demonstrating a clear sex difference, while *E. durans* was positively associated with Neurocognitive symptoms severity in males, this species was found to be negatively associated with symptom severity in females. This is a potentially important finding because *E. durans* has been studied for its probiotic potential and has been found to possess anti-inflammatory properties [87], and may also increase the known anti-inflammatory species *Faecalibacterium prausnitzii* [88]. While there is extremely limited research investigating sex differences in the function of *E. durans*, Wallis et al. [31] found sex specific associations between *Enterococcus* at the genus level and ME/CFS symptoms. Interestingly, in contrast to the findings of this paper, Wallis et al. [31] found a positive relationship between symptoms and *Enterococcus* only in females. Taken together, these somewhat contradictory findings suggest that *Enterococcus* species may interact with sex hormones in complex ways which warrant further investigation.

Alistipes shahii was found to be negatively associated with the severity of both Neurocognitive, and Stress and Anxiety symptoms, but only in males. At the genus level, *Alistipes* have been associated with psychological disorders such as depression [89, 90] and ASD [91, 92], however findings have been contradictory in terms of whether *Alistipes* are more abundant in symptomatic or healthy groups. Several hypotheses have been proposed regarding how *Alistipes* may influence symptom expression including inflammation, interference with serotonergic signalling, and metabolite production [93]. Parker et al. [94] specify a need for further research into the genus given the contradictory findings regarding its potential protective or pathogenic role. The current findings suggest that further investigations would benefit from being conducted at the species level which would provide more detailed and precise information about the protective and pathogenic potential of different members of the *Alistipes* genus. Further, the results of the current study suggest that sex differences should be considered in future studies as sex hormones may provide further insight and clarity into the role of *Alistipes* in psychological health and disease.

Demonstrating sex consistency, fungi were found to be positively associated with Neurocognitive symptom severity in both males (*Rhodotorula mucilaginosa*) and females (*Candida glabrata* and *C. parapsilosis*). This finding is consistent with previous research which has demonstrated an increased abundance of *Candida* in individuals diagnosed with neurocognitive disorders [92, 95]. It is proposed that *Rhodotorula* species in the gut are likely to provide benefits to the host through the production of various nutrients, and potentially neutralising the toxins of pathogens [96]. However, Hof [96] also proposes that *Rhodotorula* species could be detrimental in large numbers as they are able to metabolise short-chain fatty acids, thereby reducing their availability. *Rhodotorula* may also have a detrimental effect on certain pathophysiological groups (such as critically ill patients) or immunocompromised hosts. *Candida* species, including *C. glabrata* and *C. parapsilosis*, are commonly found in the human gastrointestinal and genitourinary tracts and skin [97], however, they appear to be opportunistic pathogens, having detrimental effects on immunocompromised individuals [98]. Specifically, *C. parapsilosis* has been found in greater abundance in patients with Rett syndrome and is believed to be involved in chronic inflammation [95]. As such, the results of the current study are consistent with previous research demonstrating a potential association between these *Candida* species and Neurocognitive symptom expression.

Stress and anxiety symptoms

Both stress and anxiety disorders are prevalent as clinical entities with a high disease burden, and also occur at subclinical levels in the population with considerable impairment in functioning [99, 100]. They too have been associated with changes in microbial composition of the gut [4, 6]. Results of the current study show that for females, only species belonging to the Firmicutes phylum were found to be associated with Stress and Anxiety symptom severity. *Clostridium innocuum*, *E. durans*, *E. faecium*, *L. lactis*, and *Ruminococcus gnavus* were

found to be negatively associated with Stress and Anxiety symptom severity. The finding regarding *R. gnavus* is inconsistent with Jiang et al. [4] who found an increased abundance of the species in those with GAD, however they did not investigate sex differences. In males, *L. lactis* was also found to be negatively associated with Stress and Anxiety symptom severity, demonstrating some sex consistency. *Lactobacillus vaginalis*, *Alistipes shahii*, and *Exophiala dermatitidis* were also negatively associated with Stress and Anxiety symptom severity in males.

E. dermatitidis is a black yeast that has been described as an opportunistic pathogen [101] which is typically found in domestic settings on plastics and rubbers in humid environments such as saunas and dishwashers [102]. Lavrin et al. [102] discussed the possible role of *E. dermatitidis* in neurocognitive disease such as Alzheimer's, however, their study focused on a particular strain of *E. dermatitidis* (EXF – 10,123). *E. dermatitidis* has also been associated with systemic infection [103] and cirrhosis of the liver [104]. These findings are related to circumstances where *E. dermititidis* is found outside of the gut, in some instances crossing the blood-brain-barrier into the brain. An explanation for the contrasting results between the current study and that of Lavrin et al. [102] and others who have found the microorganism to be associated with severe negative health outcomes may be that *E. dermatitidis* exerts a different influence via the BGMA as a gut microbe, as opposed to direct contact with the CNS. Given the stark contrast between the current findings and previous research regarding *E. dermatitidis*, together with the common presence of *E. detmatitidis* in household appliances such as dishwashers [105], these finding calls for further investigation.

L. plantarum and *Streptococcus gallolyticus* were positively associated with symptom severity in males, while *L. paracasei* and *S. dysgalactiae* were found to be positively associated with symptom severity in females. The finding that *Lactobacillus* species were positively related to Stress and Anxiety symptom severity is consistent with the findings of Jiang et al. [4] and somewhat consistent with Taylor et al. [6] who only found *Lactobacillus* to be positively associated with anxiety symptoms in females. *Lactobacillus* species are generally considered to be probiotic, with *L. plantarum* and *L. paracasei* both included in popular over the counter probiotic supplements. These findings are similar to several others who have demonstrated a positive association between *Lactobacillus* and the expression of various psychosocial symptoms [4, 6, 31, 86, 92, 106]. Most species belonging to the *Lactobacillus* and *Streptococcus* genera produce D-lactic acid, which may be associated with altered mental states [31, 86, 107]. Taken together, these findings bring into question whether such probiotic supplements should be used indiscriminately, let alone publicised as health promoting. Given that the current study is associative only, and information regarding probiotic use and health status was unavailable, it may be that those experiencing high levels of anxiety were taking a probiotic supplement, hence the increased abundance of these species. As such, it is not the contention of this study to suggest that *Lactobacillus* species are pathogenic, however, it does demonstrate that further research is called for.

Sleep and fatigue symptoms

Sleep plays an important role in maintaining health [108]. Many physiological and psychological disorders are associated with poor sleep [109, 110]. While having received less attention in the literature thus far, evidence shows an association between GM and sleep physiology [1, 111]. The current study adds to this literature by demonstrating sex divergent relationships between GM and severity of Sleep and Fatigue symptoms. *Lactobacillus delbrueckii* and *E. durans* were found to be positively associated with Sleep and Fatigue symptom severity in males, consistent with findings regarding Neurocognitive symptom severity. *L. delbrueckii* is also regarded as a probiotic, included in a probiotic preparation called VSL #3 [112]. While there do not appear to be any reports of *L. delbrueckii* being associated with psychological symptoms, the species has been linked to a case of pyelonephritis (kidney infection) and bacteremia [113], and urinary tract infections [114]. In females, *L. acidophilus*, also included in probiotic preparation VSL #3, was found to be positively associated with Sleep and Fatigue symptoms. These findings again draw attention to the need for further research into GM labelled as probiotics. Individuals may look to probiotics as an easy and safe supplement to improve their health, however these findings add weight to a growing body of evidence which suggest that, taken without consideration, probiotic supplements may worsen certain symptoms.

Streptococcus species were found to be negatively associated with Sleep and Fatigue symptom severity in both males (*S. mutans*) and females (*S. sanguinis*). *S. mutans* is typically regarded as a causative agent of tooth decay [115], and has also been associated with inflammatory bowel disorders [116]. *S. mutans* is able to metabolise a wide range of carbohydrates [117] which may be the mechanism through which it influences symptom expression. *S. sanguinis* is considered a commensal of the oral microbiota, but has been associated with endocarditis (infection of the valves or endocardial lining of the heart [118]) and potentially with cases of colonic carcinoma [119]. It is unclear how these species may be negatively associated with symptoms of Sleep and Fatigue in the current sample. Given the relatively small effect sizes,

this may be due to statistical artifact. However, given that *S. mutans* has been demonstrated to be able to metabolise carbohydrates, further research is warranted.

As was the case with Neurocognitive symptoms, *B. bifidum* was negatively associated with Sleep and Fatigue symptom severity in males. Conversely, unidentified *Bifidobacterium* species were found to be positively associated with symptom severity in females. This is again suggestive that species belonging to the *Bifidobacterium* genus act in a sex dependent manner [32, 80]. *E. detmatitidis* was also again found to be negatively associated with Sleep and Fatigue symptom severity in males only.

The clinical relevance of the microgenderome

The results of the current study in no way propose a causal link between GM and psychological symptom severity. However, consistent with previous research, the results provide further evidence for the microgenderome [31–33]. The pattern of relationships between GM and all four psychological symptom domains clearly varied by sex. This was despite all but one species which was related to symptom expression (*R. gnavus*) having a similar abundance in males and females. This finding was consistent with Wallis et al. [31] who also demonstrated sex-dependent relationships between GM and symptom expression despite similar GM composition. Taken together, these findings suggest that the role of sex hormones goes beyond organisational effects of GM, but also influences the action of specific species. Vemuri et al. [36] suggest that insight into the bidirectional interactions between sex hormones, GM, and immunity may provide valuable understanding of the sex discrepancies in susceptibility to psychological disorders.

Further to this point, it seems inappropriate to provide males and females with the same treatment and expect it to be equally efficacious. For example, sex differences have been noted in the efficacy of psychopharmacological treatment [120, 121]. Given that GM have been demonstrated to alter the bioavailability, bioactivity, or toxicity of drugs [122, 123] it is plausible that they may do so in a sex dependent manner. A study using a murine model found that *Ruminococcus flavefaciens* altered the effectiveness of antidepressant duloxetine. *Ruminococcus* has been found to be more abundant in females [33, 67], while *R. gnavus* was found to be more abundant in males in the current study. Although inconsistent, these findings suggest that *Ruminoccocus* could be a genus particularly vulnerable to the effect of sex hormones. These findings suggest a need for further research into the potential interactions between psychopharmacological treatments and GM in males and females. While it is not proposed that GM modulation take the place of traditional psychopharmacological treatments, the microgenderome offers valuable insight into potentially enhancing treatment outcomes through the understanding of potential sex differences in GM-drug-host relationships. Before such auxiliary treatments are introduced, however, further research is needed.

Limitations and future directions

The current study was limited by large amounts of missing data which precluded the ability to perform more sophisticated statistical analyses which could have provided a better picture of the intricate relationships between various species and symptoms. While the use of pairwise analysis was deemed necessary, this method of dealing with missing data can introduce bias in cases where data are not missing completely at random. As such, the focus of this study was not intended to be on any specific species, or suggesting that any specific species be used in the treatment of psychological disorders. Instead, the focus was more so on the pattern of relationships, and further exploring the concept of the microgenderome. Missing data also meant that the sample size available for certain correlations was small, meaning that statistical power was low. This could have undermined the true extent of the associations between GM and symptom severity. Missing data is a common issue in microbiota research and is complicated by the fact that it is unlike typical missing data. Referred to as "structural zeros", missingness in microbiological data is due to an individual's underlying biology [43]. This issue is further exacerbated by the fact that how this missingness, or structural zeros, is dealt with is not described in BGMA research studies. In a field that already has a number of different methodological procedures which may contribute to the discrepancies in previous research findings, the lack of clarity around how missingness is treated adds to potential methodological biases. It is imperative that a standardised method of dealing with missingness is developed and employed in BGMA research going forward.

A further limitation relates to the range of factors that were not controlled for, including but not limited to diet, exercise, medication, probiotic use, and underlying clinical diagnoses as this information was not available in the retrospectively collected dataset. As such, it was unclear whether any of these factors mediated the relationships found between GM and symptom severity. Future studies must make a concerted effort to control for as many extraneous variables that have been demonstrated to influence GM composition. Ideally, this should become the norm in BGMA research, as without controlling for factors that are known to influence GM, our understanding of GM-symptom associations will be incomplete. While large scale population studies remain useful

in elucidating relationships between GM and symptom expression, smaller scale, well phenotyped and controlled longitudinal studies may facilitate greater control of numerous extraneous factors.

More generally, in addition to not controlling for, or considering, the multitude of factors that may influence GM composition, studies investigating the association between GM and psychological symptoms suffer from disparate methods of both microbial (culture-based methods, antibody-based assays, ribosomal RNA (16S rDNA) and shotgun metagenomic sequencing) and symptom data collection. Culture-based methods such as the one used in the current study counts only viable (live) cells, whereas sequencing techniques based on DNA cannot distinguish between live and dead cells [124]. An important question that has received little attention is whether dead cells impact on host health. The majority of research on this particular topic is in regards to the physiological functions of live probiotic species compared to the same heat killed species, but does demonstrate that non-viable (dead) bacteria may still influence host health [125–128]. These disparities in research methods may contribute to the inconsistencies found within the literature, therefore making it difficult to directly compare the findings across studies. While it may not always be practically feasible, it has been suggested that an ideal approach would be using culture-based methods alongside sequencing techniques, where the advantages of each together could outweigh the disadvantages of a single technique alone [129–131].

There are also several sampling methods for gut microbiota including stool sampling (most common) and biopsy, among others, with Tang et al. [132] calling for more precise measures of GM that are able to be used in large scale research and in healthy controls. This will inevitably be enabled by technological advances, as evidenced by the development of smart capsules, which hold promise as non-invasive accurate measures of intestinal microbiota [132, 133]. Going forward, efforts must be made for more standardised testing procedures. There is a need for more clinical trials with strict controls on extraneous variables, investigating particular psychological conditions. Given the immense interindividual differences that exist within the GM, longitudinal and repeated measures studies may also provide valuable insight into how shifts in GM relate to changes in symptom expression within the same individual. It is only once these pathways are established that GM modulation can be seriously considered in the diagnostic and treatment process.

In addition to investigating the bacterial component of the GM, future research must also consider the influence of other members of the GM such as viruses, fungi, protozoa, and helminths. While less numerous, these microbes may still have important effects on their human host. Ganci et al. [41] have taken an initial step in broadening the scope of BGMA research by being the first to investigate the effect of two common protozoan members of the GM (*Blastocystis* sp. and *Dientamoeba fragilis*) on psychological symptom severity. While the findings showed that these protozoa did not effect psychological symptom expression, further studies are called for before conclusions can be drawn. Following the current study, the next logical step for further research would be to consider the interactions between diverse members of the GM. This is important to consider as changes in the delicate balance of the gut ecosystem can change the fitness and pathogenic potential of otherwise commensal organisms. To understand the precise mechanisms of GM, research must consider the interplay between all members of the gut ecosystem.

Conclusion

The results of the current study provide further evidence of associations between GM and psychological symptom expression in a human sample. Sex divergent patterns of these associations also provides additional support for the concept of the microgenderome. The results serve to suggest that a number of specific microbial species may hold promise in either providing auxiliary diagnostic information as potential biomarkers of health status as well as potential auxiliary treatment options. The findings of the current study also demonstrate the need for caution with regard to the indiscriminate use of probiotic supplements. Several species typically found in popular over the counter probiotic formulations were found to be positively associated with symptom severity. In some cases, the use of probiotics may do more harm than good. At this stage, it is not being suggested that probiotic use should be subject to the same restrictions as some medications, however there is a pressing need for further research into species currently considered probiotic.

The intersection of microbiology and psychology had remained largely distinct until the last decade or so [134]. This paper has demonstrated that the field of psychology can benefit from both the incorporation of GM research into the practice of clinical formulation, though also may be uniquely placed to inform microbiology research regarding the interactions between clinical symptoms and what this may mean for the GM. It is not the contention of this paper to suggest that psychologists should become well-versed in microbiology, nor is it suggested that all clients be referred to have their GM screened. To make the concept to the BGMA more accessible to practicing psychologists, Ganci et al. [135] related GM to each component of the Four P

model of case formulation. In conducting clinical interviews, psychologists should consider asking questions around possible factors that may influence GM composition (such as a person's diet or recent changes in diet), as these may be related to a client's presenting problem, and to keep in mind that this may occur in a sex specific manner. It is particularly important to consider the possible role of GM when social and emotional precipitating or perpetuating factors are not present. In such cases, a multidisciplinary approach to treatment including a microbiologist may be advantageous. Inclusion of a complementary treatment targeting the GM would be in line with the growing push for personalised medicine, with the aim of tailoring an individual client's treatment using a multidisciplinary approach which allows for a more holistic consideration of health and wellbeing. Before that is possible however, further research working towards understanding the precise mechanisms through which GM act on psychological symptom expression is needed.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12888-022-03947-7.

Additional file 1.

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All authors were involved in the concept of the study presented in the manuscript. M.G. wrote the main manuscript text. E.S. and M.B. contributed to both the study design and manuscript. Through Bioscreen, H.B. provided the data analysed. The author(s) read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available from Bioscreen but restrictions apply to the availability of these data, which were used under licence for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Bioscreen.

Declarations

Ethics approval and consent to participate
All methods were carried out in accordance with relevant guidelines and regulations. This study received ethical approval through the Victoria University Human Research Ethics Committee (HRE16–071). Retrospective data was used in the current study. When providing their data, Bioscreen patients were informed that their data may be used for research purposes, and that all information would be de-identified. Patients had the option to "opt out" by checking a box on the Bioscreen patient questionnaire (BPQ). By completing the BPQ, and not checking the "opt out" box, informed consent was provided. If the questionnaire was not completed, or patients checked the "opt out" box, consent was not provided, and their data was not used.

Consent for publication
The manuscript does not include details, images, or videos relating to an individual person.

Competing interests
The Authors declare that there are no competing interests.

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References

1. Anderson J, Carroll I, Azcarate-Peril M, et al. A preliminary examination of gut microbiota, sleep, and cognitive flexibility in healthy older adults. Sleep Med. 2017;38:104–7. https://doi.org/10.1016/j.sleep.2017.07.018.
2. Chen Y, Bai J, Wu D, et al. Association between fecal microbiota and generalized anxiety disorder: severity and early treatment response. J Affect Disord. 2019;259:56–66. https://doi.org/10.1016/j.jad.2019.08.014.
3. Jiang H, Ling Z, Zhang Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav Immun. 2015;48:186–94. https://doi.org/10.1016/j.bbi.2015.03.016.
4. Jiang H, Zhang X, Yu Z, et al. Altered gut microbiota profile in patients with generalized anxiety disorder. J Psychiatr Res. 2018;104:130–6. https://doi.org/10.1016/j.jpsychires.2018.07.007.
5. Lai W, Deng W, Xu S, et al. Shotgun metagenomics reveals both taxonomic and tryptophan pathway differences of gut microbiota in major depressive disorder patients. Psychol Med. 2019;51(1):90–101. https://doi.org/10.1017/s0033291719003027.
6. Taylor A, Thompson S, Edwards C, Musaad S, Khan N, Holscher H. Associations among diet, the gastrointestinal microbiota, and negative emotional states in adults. Nutr Neurosci. 2019;23(12):983–92. https://doi.org/10.1080/1028415x.2019.1582578.
7. Rong H, Xie X, Zhao J, et al. Similarly in depression, nuances of gut microbiota: evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients. J Psychiatr Res. 2019;113:90–9. https://doi.org/10.1016/j.jpsychires.2019.03.017.
8. Zhou Y, Chen C, Yu H, Yang Z. Fecal microbiota changes in patients with postpartum depressive disorder. Front Cell Infect Microbiol. 2020;10. https://doi.org/10.3389/fcimb.2020.567268.
9. Cheng L, Liu Y, Wu C, Wang S, Tsai Y. Psychobiotics in mental health, neurodegenerative and neurodevelopmental disorders. J Food Drug Anal. 2019;27(3):632–48. https://doi.org/10.1016/j.jfda.2019.01.002.
10. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474(11):1823–36. https://doi.org/10.1042/bcj20160510.
11. Matijašić M, Meštrović T, Čipčić Paljetak H, Perić M, Barešić A, Verbanac D. Gut microbiota beyond bacteria—mycobiome, virome, archaeome, and eukaryotic parasites in IBD. Int J Mol Sci. 2020;21(8):2668. https://doi.org/10.3390/ijms21082668.
12. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol. 2015;28(2):203–9.
13. Clapp M, Aurora N, Herrera L, Bhatia M, Wilen E, Wakefield S. Gut Microbiota's effect on mental health: the gut-brain Axis. Clin Pract. 2017;7(4):987. https://doi.org/10.4081/cp.2017.987.
14. Groen R, de Clercq N, Nieuwdorp M, Hoenders H, Groen A. Gut microbiota, metabolism and psychopathology: a critical review and novel perspectives. Crit Rev Clin Lab Sci. 2018;55(4):283–93. https://doi.org/10.1080/10408363.2018.1463507.
15. King C, Desai H, Sylvetsky A, et al. Baseline human gut microbiota profile in healthy people and standard reporting template. Plos One. 2019;14(9):e0206484. https://doi.org/10.1371/journal.pone.0206484.

16. Quigley EM. Gut bacteria in health and disease. Gastroenterol Hepatol. 2013;9(9):560–9.
17. Rea K, Dinan T, Cryan J. Gut microbiota: a perspective for psychiatrists. Neuropsychobiology. 2019;79(1):50–62. https://doi.org/10.1159/000504495.
18. Chen L, Abbaspour A, Mkoma G, Bulik C, Rück C, Djurfeldt D. Gut microbiota in psychiatric disorders: a systematic review. Psychosom Med. 2021;83(7):679–92. https://doi.org/10.1097/psy.0000000000000959.
19. Cheung S, Goldenthal A, Uhlemann A, Mann J, Miller J, Sublette M. Systematic review of gut microbiota and major depression. Front Psychiatry. 2019;10. https://doi.org/10.3389/fpsyt.2019.00034.
20. McGuinness AJ, Davis JA, Dawson SL, et al. A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia [published online ahead of print, 2022 Feb 22]. Mol Psychiatry. 2022. https://doi.org/10.1038/s41380-022-01456-3.
21. Nikolova V, Hall M, Hall L, Cleare A, Stone J, Young A. Perturbations in gut microbiota composition in psychiatric disorders. JAMA Psychiatry. 2021;78(12):1343. https://doi.org/10.1001/jamapsychiatry.2021.2573.
22. Simpson CA, Diaz-Arteche C, Eliby D, Schwartz OS, Simmons JG, Cowan CSM. The gut microbiota in anxiety and depression - a systematic review. Clin Psychol Rev. 2021;83:101943. https://doi.org/10.1016/j.cpr.2020.101943.
23. Bharti R, Grimm D. Current challenges and best-practice protocols for microbiome analysis. Brief Bioinform. 2019;22(1):178–93. https://doi.org/10.1093/bib/bbz155.
24. Hiergeist A, Gläsner J, Reischl U, Gessner A. Analyses of intestinal microbiota: culture versus sequencing: figure 1. ILAR J. 2015;56(2):228–40. https://doi.org/10.1093/ilar/ilv017.
25. Rezasoltani S, Ahmadi Bashirzadeh D, Nazemalhosseini Mojarad E, Asadzadeh Aghdaei H, Norouzinia M, Shahrokh S. Signature of gut microbiome by conventional and advanced analysis techniques: advantages and disadvantages. Middle East J Dig Dis. 2019;12(1):5–11. https://doi.org/10.15171/mejdd.2020.157.
26. Capone I, Marchetti P, Ascierto P, Malorni W, Gabriele L. Sexual dimorphism of immune responses: a new perspective in Cancer immunotherapy. Front Immunol. 2018;9. https://doi.org/10.3389/fimmu.2018.00552.
27. Martin R, Makino H, Cetinyurek Yavuz A, et al. Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. Plos One. 2016;11(6):e0158498. https://doi.org/10.1371/journal.pone.0158498.
28. Ma Z, Li W. How and why men and women differ in their microbiomes: medical ecology and network analyses of the microgenderome. Adv Sci. 2019;6(23):1902054. https://doi.org/10.1002/advs.201902054.
29. Clarke G, Grenham S, Scully P, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol Psychiatry. 2012;18(6):666–73. https://doi.org/10.1038/mp.2012.77.
30. Mulak A, Taché Y, Larauche M. Sex hormones in the modulation of irritable bowel syndrome. World J Gastroenterol. 2014;20(10):2433. https://doi.org/10.3748/wjg.v20.i10.2433.
31. Wallis A, Butt H, Ball M, Lewis D, Bruck D. Support for the Microgenderome: associations in a human clinical population. Sci Rep. 2016;6(1). https://doi.org/10.1038/srep19171.
32. Chen J, Zheng P, Liu Y, et al. Sex differences in gut microbiota in patients with major depressive disorder. Neuropsychiatr Dis Treat. 2018;14:647–55. https://doi.org/10.2147/ndt.s159322.
33. Gao X, Zhang M, Xue J, et al. Body mass index differences in the gut microbiota are gender specific. Front Microbiol. 2018;9. https://doi.org/10.3389/fmicb.2018.01250.
34. Mueller S, Saunier K, Hanisch C, et al. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. Appl Environ Microbiol. 2006;72(2):1027–33. https://doi.org/10.1128/aem.72.2.1027-1033.2006.
35. Singh P, Manning S. Impact of age and sex on the composition and abundance of the intestinal microbiota in individuals with and without enteric infections. Ann Epidemiol. 2016;26(5):380–5. https://doi.org/10.1016/j.annepidem.2016.03.007.
36. Vemuri R, Sylvia K, Klein S, et al. The microgenderome revealed: sex differences in bidirectional interactions between the microbiota, hormones, immunity and disease susceptibility. Semin Immunopathol. 2018;41(2):265–75. https://doi.org/10.1007/s00281-018-0716-7.
37. Haro C, Rangel-Zúñiga O, Alcalá-Díaz J, et al. Intestinal microbiota is influenced by gender and body mass index. Plos One. 2016;11(5):e0154090. https://doi.org/10.1371/journal.pone.0154090.
38. Severance E, Gressitt K, Stallings C, et al. Candida albicans exposures, sex specificity and cognitive deficits in schizophrenia and bipolar disorder. NPJ Schizophr. 2016;2(1). https://doi.org/10.1038/npjschz.2016.18.
39. Moloney R, Desbonnet L, Clarke G, Dinan T, Cryan J. The microbiome: stress, health and disease. Mamm Genome. 2013;25(1–2):49–74. https://doi.org/10.1007/s00335-013-9488-5.
40. Coulson S, Butt H, Vecchio P, Gramotnev H, Vitetta L. Green-lipped mussel extract (Perna canaliculus) and glucosamine sulphate in patients with knee osteoarthritis: therapeutic efficacy and effects on gastrointestinal microbiota profiles. Inflammopharmacology. 2012;21(1):79–90. https://doi.org/10.1007/s10787-012-0146-4.
41. Ganci M, Butt H, Tyrrell J, Suleyman E, Ball M. The effect of Blastocystis sp. and Dientamoeba fragilis on psychological symptom severity in a sample of clinically diverse males and females. Curr Psychol. 2021. https://doi.org/10.1007/s12144-021-01700-z.
42. Jakobsen J, Gluud C, Wetterslev J, Winkel P. When and how should multiple imputation be used for handling missing data in randomised clinical trials – a practical guide with flowcharts. BMC Med Res Methodol. 2017;17(1). https://doi.org/10.1186/s12874-017-0442-1.
43. Kaul A, Davidov O, Peddada S. Structural zeros in high-dimensional data with applications to microbiome studies. Biostatistics. 2017;kxw053. https://doi.org/10.1093/biostatistics/kxw053.
44. Rana R, Singhal R, Dua P. Deciphering the dilemma of parametric and nonparametric tests. J Pract Cardiovasc Sci. 2016;2(2):95. https://doi.org/10.4103/2395-5414.191521.
45. Nahm F. Nonparametric statistical tests for the continuous data: the basic concept and the practical use. Korean J Anesthesiol. 2016;69(1):8. https://doi.org/10.4097/kjae.2016.69.1.8.
46. Derrick B, White P, Toher D. Parametric and non-parametric tests for the comparison of two samples which both include paired and unpaired observations. J Mod Appl Stat Methods. 2020;18(1):2–23. https://doi.org/10.22237/jmasm/1556669520.
47. Happ M, Bathke A, Brunner E. Optimal sample size planning for the Wilcoxon-Mann-Whitney test. Stat Med. 2018;38(3):363–75. https://doi.org/10.1002/sim.7983.
48. Fay M, Proschan M. Wilcoxon-Mann-Whitney or t-test? On assumptions for hypothesis tests and multiple interpretations of decision rules. Stat Surv. 2010;4(none). https://doi.org/10.1214/09-ss051.
49. Button K, Ioannidis J, Mokrysz C, et al. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci. 2013;14(5):365–76. https://doi.org/10.1038/nrn3475.
50. Faber J, Fonseca L. How sample size influences research outcomes. Dental Press J Orthod. 2014;19(4):27–9. https://doi.org/10.1590/2176-9451.19.4.027-029.ebo.
51. Vadillo M, Konstantinidis E, Shanks D. Underpowered samples, false negatives, and unconscious learning. Psychon Bull Rev. 2015;23(1):87–102. https://doi.org/10.3758/s13423-015-0892-6.
52. Rubin M. Do p values lose their meaning in exploratory analyses? It depends how you define the familywise error rate. Rev Gen Psychol. 2017;21(3):269–75. https://doi.org/10.1037/gpr0000123.
53. Akoglu H. User's guide to correlation coefficients. Turk J Emerg Med. 2018;18(3):91–3. https://doi.org/10.1016/j.tjem.2018.08.001.
54. Long J, Cliff N. Confidence intervals for Kendall's tau. Br J Math Stat Psychol. 1997;50(1):31–41. https://doi.org/10.1111/j.2044-8317.1997.tb01100.x.
55. Walker D. JMASM9: converting Kendall's tau for correlational or meta-analytic analyses. J Mod Appl Stat Methods. 2003;2(2):525–30. https://doi.org/10.22237/jmasm/1067646360.
56. Cohen J. Statistical power analysis for the behavioral sciences. Hillsdale: L. Erlbaum Associates; 1988.
57. McGrath R, Meyer G. When effect sizes disagree: the case of r and d. Psychol Methods. 2006;11(4):386–401. https://doi.org/10.1037/1082-989x.11.4.386.

58. Abate K. Gender disparity in prevalence of depression among patient population: a systematic review. Ethiop J Health Sci. 2013;23(3). https://doi.org/10.4314/ejhs.v23i3.11.
59. Barsky AJ, Peekna HM, Borus JF. Somatic symptom reporting in women and men. J Gen Intern Med. 2001;16(4):266–75. https://doi.org/10.1046/j.1525-1497.2001.00229.x.
60. Laws K, Irvine K, Gale T. Sex differences in Alzheimer's disease. Curr Opin Psychiatry. 2018;31(2):133–9. https://doi.org/10.1097/yco.0000000000000401.
61. McLean C, Asnaani A, Litz B, Hofmann S. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. J Psychiatr Res. 2011;45(8):1027–35. https://doi.org/10.1016/j.jpsychires.2011.03.006.
62. Seney M, Sibille E. Sex differences in mood disorders: perspectives from humans and rodent models. Biol Sex Differ. 2014;5(1). https://doi.org/10.1186/s13293-014-0017-3.
63. Solomon M, Herman J. Sex differences in psychopathology: of gonads, adrenals and mental illness. Physiol Behav. 2009;97(2):250–8. https://doi.org/10.1016/j.physbeh.2009.02.033.
64. Wickens M, Bangasser D, Briand L. Sex differences in psychiatric disease: a focus on the glutamate system. Front Mol Neurosci. 2018;11. https://doi.org/10.3389/fnmol.2018.00197.
65. Bekhbat M, Neigh G. Sex differences in the neuro-immune consequences of stress: focus on depression and anxiety. Brain Behav Immun. 2018;67:1–12. https://doi.org/10.1016/j.bbi.2017.02.006.
66. Rainville J, Hodes G. Inflaming sex differences in mood disorders. Neuropsychopharmacology. 2018;44(1):184–99. https://doi.org/10.1038/s41386-018-0124-7.
67. Org E, Mehrabian M, Parks B, et al. Sex differences and hormonal effects on gut microbiota composition in mice. Gut Microbes. 2016;7(4):313–22. https://doi.org/10.1080/19490976.2016.1203502.
68. Guo P, Zhang K, Ma X, He P. Clostridium species as probiotics: potentials and challenges. J Anim Sci Biotechnol. 2020;11(1). https://doi.org/10.1186/s40104-019-0402-1.
69. Lopetuso L, Scaldaferri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. Gut Pathog. 2013;5(1):23. https://doi.org/10.1186/1757-4749-5-23.
70. World Health Organization. Depression, vol. 2020: World Health Organization; 2020. https://www.who.int/news-room/fact-sheets/detail/depression. Accessed 26 April 2021
71. Liang S, Wu X, Hu X, Wang T, Jin F. Recognizing depression from the microbiota–gut–brain axis. Int J Mol Sci. 2018;19(6):1592. https://doi.org/10.3390/ijms19061592.
72. Luerce T, Gomes-Santos A, Rocha C, et al. Anti-inflammatory effects of Lactococcus lactis NCDO 2118 during the remission period of chemically induced colitis. Gut Pathog. 2014;6(1). https://doi.org/10.1186/1757-4749-6-33.
73. Mei H, Liu Y, Chiang Y, et al. Immunomodulatory activity ofLactococcus lactisA17 from Taiwan fermented cabbage in OVA-sensitized BALB/c mice. Evid Based Complement Alternat Med. 2013;2013:1–11. https://doi.org/10.1155/2013/287803.
74. Nuryshev M, Stoyanova L, Netrusov A. New probiotic culture of Lactococcus lactis ssp. lactis: effective opportunities and prospects. J Microb Biochem Technol. 2016;8(4). https://doi.org/10.4172/1948-5948.1000299.
75. Rodríguez-Figueroa J, González-Córdova A, Astiazaran-García H, Vallejo-Cordoba B. Hypotensive and heart rate-lowering effects in rats receiving milk fermented by specific Lactococcus lactis strains. Br J Nutr. 2012;109(5):827–33. https://doi.org/10.1017/s0007114512002115.
76. Gomi A, Iino T, Nonaka C, Miyazaki K, Ishikawa F. Health benefits of fermented milk containing Bifidobacterium bifidum YIT 10347 on gastric symptoms in adults. J Dairy Sci. 2015;98(4):2277–83. https://doi.org/10.3168/jds.2014-9158.
77. O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. Front Microbiol. 2016;7. https://doi.org/10.3389/fmicb.2016.00925.
78. Turroni F, Duranti S, Milani C, Lugli G, van Sinderen D, Ventura M. Bifidobacterium bifidum: a key member of the early human gut microbiota. Microorganisms. 2019;7(11):544. https://doi.org/10.3390/microorganisms7110544.
79. Aizawa E, Tsuji H, Asahara T, et al. Possible association of Bifidobacterium and Lactobacillus in the gut microbiota of patients with major depressive disorder. J Affect Disord. 2016;202:254–7. https://doi.org/10.1016/j.jad.2016.05.038.
80. Luk B, Veeraragavan S, Engevik M, et al. Postnatal colonization with human "infant-type" Bifidobacterium species alters behavior of adult gnotobiotic mice. PLoS One. 2018;13(5):e0196510. https://doi.org/10.1371/journal.pone.0196510.
81. Roshchina V. New trends and perspectives in the evolution of neurotransmitters in microbial, plant, and animal cells. Microb Endocrinol. 2016:25–77. https://doi.org/10.1007/978-3-319-20215-0_2.
82. Liu Y, Zhang L, Wang X, et al. Similar fecal microbiota signatures in patients with diarrhea-predominant irritable bowel syndrome and patients with depression. Clin Gastroenterol Hepatol. 2016;14(11):1602–1611.e5. https://doi.org/10.1016/j.cgh.2016.05.033.
83. Feinkohl I, Janke J, Hadzidiakos D, et al. Associations of the metabolic syndrome and its components with cognitive impairment in older adults. BMC Geriatr. 2019;19(1). https://doi.org/10.1186/s12877-019-1073-7.
84. Harvey PD. Mood symptoms, cognition, and everyday functioning: in major depression, bipolar disorder, and schizophrenia. Innov Clin Neurosci. 2011;8(10):14–8.
85. Perini G, Cotta Ramusino M, Sinforiani E, Bernini S, Petrachi R, Costa A. Cognitive impairment in depression: recent advances and novel treatments. Neuropsychiatr Dis Treat. 2019;15:1249–58. https://doi.org/10.2147/ndt.s199746.
86. Rao S, Rehman A, Yu S, de Andino N. Brain fogginess, gas and bloating: a link between SIBO, probiotics and metabolic acidosis. Clin Transl Gastroenterol. 2018;9(6):e162. https://doi.org/10.1038/s41424-018-0030-7.
87. Avram-Hananel L, Stock J, Parlesak A, Bode C, Schwartz B. E Durans strain M4–5 isolated from human colonic Flora attenuates intestinal inflammation. Dis Colon Rectum. 2010;53(12):1676–86. https://doi.org/10.1007/dcr.0b013e3181f4b148.
88. Carasi P, Racedo S, Jacquot C, Elie A, Serradell M, Urdaci M. Enterococcus durans EP1 a promising anti-inflammatory probiotic able to stimulate sIgA and to increase Faecalibacterium prausnitzii abundance. Front Immunol. 2017;8. https://doi.org/10.3389/fimmu.2017.00088.
89. Naseribafrouei A, Hestad K, Avershina E, et al. Correlation between the human fecal microbiota and depression. Neurogastroenterol Motil. 2014;26(8):1155–62. https://doi.org/10.1111/nmo.12378.
90. Zheng P, Zeng B, Zhou C, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol Psychiatry. 2016;21(6):786–96. https://doi.org/10.1038/mp.2016.44.
91. De Angelis M, Piccolo M, Vannini L, et al. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. PLoS One. 2013;8(10):e76993. https://doi.org/10.1371/journal.pone.0076993.
92. Strati F, Cavalieri D, Albanese D, et al. New evidences on the altered gut microbiota in autism spectrum disorders. Microbiome. 2017;5(1). https://doi.org/10.1186/s40168-017-0242-1.
93. Dhaliwal G. Alistipes: the influence of a commensal on anxiety and depression. Catalyst. 2019;3(1):1–9.
94. Parker B, Wearsch P, Veloo A, Rodriguez-Palacios A. The genus alistipes: gut bacteria with emerging implications to inflammation, cancer, and mental health. Front Immunol. 2020;11. https://doi.org/10.3389/fimmu.2020.00906.
95. Strati F, Calabrò A, Donati C, et al. Intestinal Candida parapsilosis isolates from Rett syndrome subjects bear potential virulent traits and capacity to persist within the host. BMC Gastroenterol. 2018;18(1). https://doi.org/10.1186/s12876-018-0785-z.
96. Hof H. Rhodotorula spp. in the gut - foe or friend? GMS Infect Dis. 2019;7:1–6.
97. Kabir M, Ahmad Z. Candida infections and their prevention. ISRN Prev Med. 2013;2013:1–13. https://doi.org/10.5402/2013/763628.
98. Alves R, Kastora S, Gomes-Gonçalves A, et al. Transcriptional responses of Candida glabrata biofilm cells to fluconazole are modulated by the carbon source. NPJ Biofilms Microb. 2020;6(1). https://doi.org/10.1038/s41522-020-0114-5.

99. Bandelow B, Michaelis S. Epidemiology of anxiety disorders in the 21st century. Anxiety. 2015;17(3):327–35. https://doi.org/10.31887/dcns.2015.17.3/bbandelow.
100. Sussams R, Schlotz W, Clough Z, et al. Psychological stress, cognitive decline and the development of dementia in amnestic mild cognitive impairment. Sci Rep. 2020;10(1). https://doi.org/10.1038/s41598-020-60607-0.
101. Kirchhoff L, Olsowski M, Rath P, Steinmann J. Exophiala dermatitidis: key issues of an opportunistic fungal pathogen. Virulence. 2019;10(1):984–98. https://doi.org/10.1080/21505594.2019.1596504.
102. Lavrin T, Konte T, Kostanjšek R, et al. The neurotropic black yeast Exophiala dermatitidis induces Neurocytotoxicity in neuroblastoma cells and progressive cell death. Cells. 2020;9(4):963. https://doi.org/10.3390/cells9040963.
103. Oztas E, Odemis B, Kekilli M, et al. Systemic phaeohyphomycosis resembling primary sclerosing cholangitis caused by Exophiala dermatitidis. J Med Microbiol. 2009;58(9):1243–6. https://doi.org/10.1099/jmm.0.008706-0.
104. Hong K, Kim J, Jang S, Yu E, Kim E. Liver cirrhosis caused by Exophiala dermatitidis. J Med Microbiol. 2009;58(5):674–7. https://doi.org/10.1099/jmm.0.002188-0.
105. Zupančič J, Novak Babič M, Zalar P, Gunde-Cimerman N. The black yeast Exophiala dermatitidis and other selected opportunistic human fungal pathogens spread from dishwashers to kitchens. Plos One. 2016;11(2):e0148166. https://doi.org/10.1371/journal.pone.0148166.
106. He Y, Kosciolek T, Tang J, et al. Gut microbiome and magnetic resonance spectroscopy study of subjects at ultra-high risk for psychosis may support the membrane hypothesis. Eur Psychiatry. 2018;53:37–45. https://doi.org/10.1016/j.eurpsy.2018.05.011.
107. Sheedy J, Wettenhall R, Scanlon D, et al. Increased d-lactic acid intestinal bacteria in patients with chronic fatigue syndrome. In Vivo (Brooklyn). 2009;23(4):621–8.
108. Czeisler C. Duration, timing and quality of sleep are each vital for health, performance and safety. Sleep Health. 2015;1(1):5–8. https://doi.org/10.1016/j.sleh.2014.12.008.
109. Sharma S, Kavuru M. Sleep and metabolism: an overview. Int J Endocrinol. 2010;2010:1–12. https://doi.org/10.1155/2010/270832.
110. Worley S. The extraordinary importance of sleep: the detrimental effects of inadequate sleep on health and public safety drive an explosion of sleep research. J Formul Manag. 2018;43(12):758–63.
111. Smith R, Easson C, Lyle S, et al. Gut microbiome diversity is associated with sleep physiology in humans. Plos One. 2019;14(10):e0222394. https://doi.org/10.1371/journal.pone.0222394.
112. Bambury A, Sandhu K, Cryan J, Dinan T. Finding the needle in the haystack: systematic identification of psychobiotics. Br J Pharmacol. 2018;175(24):4430–8. https://doi.org/10.1111/bph.14127.
113. DuPrey K, McCrea L, Rabinowitch B, Azad K. Pyelonephritis and bacteremia fromLactobacillus delbrueckii. Case Rep Infect Dis. 2012;2012:1–3. https://doi.org/10.1155/2012/745743.
114. Maillet F, Passeron A, Podglajen I, Ranque B, Pouchot J. Lactobacillus delbrueckii urinary tract infection in a male patient. Med Mal Infect. 2019;49(3):226–8. https://doi.org/10.1016/j.medmal.2018.11.006.
115. Lemos J, Palmer S, Zeng L, et al. The biology of streptococcus mutans. Microbiol Spectr. 2019;7(1). https://doi.org/10.1128/microbiolspec.gpp3-0051-2018.
116. Yoneda M, Suzuki N, Morita H, Hirofuji T. Oral Bacteria and bowel diseases – Mini review. J Gastrointest Dig Syst. 2016;06(02). https://doi.org/10.4172/2161-069x.1000404.
117. Moye Z, Zeng L, Burne R. Fueling the caries process: carbohydrate metabolism and gene regulation by Streptococcus mutans. J Oral Microbiol. 2014;6(1):24878. https://doi.org/10.3402/jom.v6.24878.
118. Zhu B, Macleod L, Kitten T, Xu P. Streptococcus sanguinis biofilm formation & interaction with oral pathogens. Future Microbiol. 2018;13(8):915–32. https://doi.org/10.2217/fmb-2018-0043.
119. Nijjer S, Dubrey S. Streptococcus sanguis endocarditis associated with colonic carcinoma. Case Rep. 2010;2010. https://doi.org/10.1136/bcr.09.2009.2311.
120. Bolea-Alamanac B, Bailey S, Lovick T, Scheele D, Valentino R. Female psychopharmacology matters! Towards a sex-specific psychopharmacology. J Psychopharmacol. 2018;32(2):125–33. https://doi.org/10.1177/0269881117747578.
121. Sramek J, Murphy M, Cutler N. Sex differences in the psychopharmacological treatment of depression. Sex Diff. 2016;18(4):447–57. https://doi.org/10.31887/dcns.2016.18.4/ncutler.
122. Walsh J, Griffin B, Clarke G, Hyland N. Drug-gut microbiota interactions: implications for neuropharmacology. Br J Pharmacol. 2018;175(24):4415–29. https://doi.org/10.1111/bph.14366.
123. Weersma R, Zhernakova A, Fu J. Interaction between drugs and the gut microbiome. Gut. 2020;69(8):1510–9. https://doi.org/10.1136/gutjnl-2019-320204.
124. Cangelosi G, Meschke J. Dead or alive: molecular assessment of microbial viability. Appl Environ Microbiol. 2014;80(19):5884–91. https://doi.org/10.1128/aem.01763-14.
125. Adams C. The probiotic paradox: live and dead cells are biological response modifiers. Nutr Res Rev. 2010;23(1):37–46. https://doi.org/10.1017/s0954422410000090.
126. Maehata H, Arai S, Iwabuchi N, Abe F. Immuno-modulation by heat-killed Lacticaseibacillus paracasei MCC1849 and its application to food products. Int J Immunopathol Pharmacol. 2021;35:205873842110082. https://doi.org/10.1177/20587384211008291.
127. Piqué N, Berlanga M, Miñana-Galbis D. Health benefits of heat-killed (Tyndallized) probiotics: an overview. Int J Mol Sci. 2019;20(10):2534. https://doi.org/10.3390/ijms20102534.
128. Sugahara H, Yao R, Odamaki T, Xiao J. Differences between live and heat-killed bifidobacteria in the regulation of immune function and the intestinal environment. Benef Microbes. 2017;8(3):463–72. https://doi.org/10.3920/bm2016.0158.
129. Al-Awadhi H, Dashti N, Khanafer M, Al-Mailem D, Ali N, Radwan S. Bias problems in culture-independent analysis of environmental bacterial communities: a representative study on hydrocarbonoclastic bacteria. Springerplus. 2013;2(1). https://doi.org/10.1186/2193-1801-2-369.
130. Fenske G, Ghimire S, Antony L, Christopher-Hennings J, Scaria J. Integration of culture-dependent and independent methods provides a more coherent picture of the pig gut microbiome. FEMS Microbiol Ecol. 2020;96(3). https://doi.org/10.1093/femsec/fiaa022.
131. Zampieri A, Babbucci M, Carraro L, Milan M, Fasolato L, Cardazzo B. Combining culture-dependent and culture-independent methods: new methodology insight on the Vibrio Community of Ruditapes philippinarum. Foods. 2021;10(6):1271. https://doi.org/10.3390/foods10061271.
132. Tang Q, Jin G, Wang G, et al. Current sampling methods for gut microbiota: a call for more precise devices. Front Cell Infect Microbiol. 2020;10. https://doi.org/10.3389/fcimb.2020.00151.
133. Waimin J, Nejati S, Jiang H, et al. Smart capsule for non-invasive sampling and studying of the gastrointestinal microbiome. RSC Adv. 2020;10(28):16313–22. https://doi.org/10.1039/c9ra10986b.
134. Zyoud S, Smale S, Waring W, Sweileh W, Al-Jabi S. Global research trends in the microbiome related to irritable bowel syndrome: a bibliometric and visualized study. World J Gastroenterol. 2021;27(13):1341–53. https://doi.org/10.3748/wjg.v27.i13.1341.
135. Ganci M, Suleyman E, Butt H, Ball M. The role of the brain–gut–microbiota axis in psychology: the importance of considering gut microbiota in the development, perpetuation, and treatment of psychological disorders. Brain Behav. 2019;9(11). https://doi.org/10.1002/brb3.1408.

Publisher's Note

Supplemental Material 1

Presented in Table 1 are the Kendall's Tau-b (τ_b) values and the associated *r*, r^2, *n*, Fisher's *z* transformation (z_r) and *p* values for all significant associations between gut microbiota species and symptom severity.

Table 1.

Conversion of Tau values to r values.

Microorganism	Tau	*r*	Z_r	*n*	*Z*obs	*r* square	*p*
Depressive symptoms (males)							
C. ramosum	0.184	0.285	0.586	65		0.081	0.036
L. lactis	-0.446	-0.645	-1.532	13		0.416	0.034
S. mutans	-0.097	-0.152	-0.306	220		0.023	0.036
B. vulgatus	-0.057	-0.089	-0.179	700		0.008	0.029
B. bifidum	-0.207	-0.319	-0.662	90		0.102	0.005
C. minutissimum	-0.299	-0.453	-0.976	30		0.205	0.025
Depressive symptoms (females)							
C. innocum	-0.103	-0.161	-0.325	259		0.026	0.016
C. citroniae	0.439	0.636	1.504	19		0.405	0.009
C. hathewayi	-0.157	-0.244	-0.498	82		0.060	0.041
E. avium	0.136	0.212	0.431	102		0.045	0.047
E. limosum	0.095	0.149	0.300	207		0.022	0.046
L. ruminis	-0.246	-0.377	-0.793	38		0.142	0.035
S. dysgalactiae	0.259	0.396	0.837	56		0.157	0.006
B. massiliensis	-0.11	-0.172	-0.347	237		0.030	0.014
C. aurimucosum	0.12	0.187	0.379	168		0.035	0.024
R. dentocariosa	0.12	0.187	0.379	140		0.035	0.04
A. oris	0.24	0.368	0.773	48		0.136	0.019
Neurocognitive symptoms (males)							
C. tertium	0.205	0.331	0.688	62		0.110	0.021
E. durans	0.448	0.680	1.658	17		0.462	0.013
L. delbrueckii	0.357	0.550	1.237	25		0.303	0.014
L. lactis	-0.543	-0.852	-2.527	13		0.726	0.012
A. shahii	-0.17	-0.265	-0.543	119		0.070	0.007
B. bifidum	-0.172	-0.264	-0.541	88		0.070	0.02
R. mucilaginosa	0.393	0.562	1.272	18		0.316	0.025
Neurocognitive symptoms (females)							
E. durans	-0.195	-0.308	-0.637	85		0.095	0.009
E. coli	-0.027	-0.042	-0.084	3006		0.002	0.028
K. pnumoniae	-0.085	-0.125	-0.251	395		0.016	0.013
C. parapsilosis	0.106	0.167	0.337	339		0.028	0.005

C. glabatra	0.22	0.339	0.705	50		0.115	0.027
Sex comaprison of E. durans					7.936		
Stress and Anxiety (males)							
L. plantarum	0.164	0.255	0.521	74		0.065	0.045
L. vaginalis	-0.385	-0.569	-1.291	20		0.323	0.021
L. lactis	-0.439	-0.636	-1.504	13		0.405	0.045
S. gallolyticus	0.487	0.693	1.706	19		0.480	0.006
A. shahii	-0.15	-0.233	-0.476	119		0.054	0.019
E. dermatitidis	-0.506	-0.714	-1.790	11		0.509	0.037
Stress and Anxiety (females)							
C. innocum	-0.09	-0.141	-0.284	252		0.020	0.038
E. durans	-0.195	-0.302	-0.622	83		0.091	0.011
E. faecium	-0.049	-0.077	-0.154	785		0.006	0.044
L. paracasei	0.069	0.108	0.217	392		0.012	0.046
L. lactis	-0.275	-0.419	-0.892	28		0.175	0.043
R. gnavus	-0.158	-0.246	-0.502	110		0.060	0.017
S. dysgalactiae	0.212	0.327	0.679	58		0.107	0.02
Sex comparison of L. lactis					-1.634		
Sleep and Fatigue (males)							
L. delbrueckii	0.338	0.506	1.116	26		0.256	0.018
E. avium	-0.246	-0.377	-0.793	33		0.142	0.048
E. durans	0.378	0.559	1.264	18		0.313	0.03
S. mutans	-0.102	-0.160	-0.322	224		0.025	0.026
B. massiliensis	0.135	0.210	0.427	91		0.044	0.035
B. bifidum	-0.249	-0.381	-0.803	90		0.145	0.001
E. dermatitidis	-0.629	-0.835	-2.408	10		0.697	0.012
Sleep and Fatigue (females)							
C. hathewayi	-0.165	-0.256	-0.524	82		0.066	0.032
L. acidophilus	0.068	0.107	0.214	472		0.011	0.03
S. sanguinis	-0.183	-0.284	-0.583	93		0.080	0.011
Bifidobacterium sp.	0.18	0.279	0.573	64		0.078	0.039
P. acnes	0.26	0.397	0.841	33		0.158	0.037

Chapter 7: General Discussion

7.1 Summary

The aim of the current study was to offer a turning point to position the BGMA as falling within the purview of psychologists. This endeavour necessitated a theoretical review, which was then supported by observational findings relating psychological symptoms with members of the gut ecosystem (protozoa, fungi, and bacteria). The concept of the microgenderome was explored by investigating these associations in a sex-specific manner. The papers presented within this thesis demonstrate the value that microbiology has to offer to the discipline of psychology in challenging traditional conceptualisations of psychological symptoms and disorders.

Paper 1 presents a theoretical rationale, incorporating current literature regarding the associations between GM and psychological symptom expression from the perspective of the Four P model of case formulation typically used in psychological practice. In doing so, this thesis approached the investigation of the BGMA from a predominantly psychological perspective, contextualising the findings in a way that is meaningful, accessible, and relevant to practicing psychologists. This paper demonstrates;

- The utility of considering GM in each stage of an individual's psychological case formulation, in addition to consideration of typical social and emotional aetiological factors in the onset, maintenance, and reduction of clinical symptoms.
- Additionally, this paper provides a context for a paradigm shift in psychology from the current CNS-centric approach to a more holistic perspective of human nature. The paper's main contention is that ongoing ambivalence toward a holistic mind-body consideration, particularly of GM factors, is at the detriment of psychological practice. Such consideration implores the collaboration between microbiology and psychology in future clinical care.

Following this review, two observational cross-sectional studies were conducted, to support the theoretical propositions of paper 1. It was evident that provision of support for the role of GM in psychological symptoms expression required an initial step to clarify the way in which the contents of the GM are considered. Thus, paper 2 addressed the role of protozoa to clarify the necessity for inclusion or exclusion of these microbes in case formulation.

Paper 2 addressed a gap in BGMA research by being the first study to investigate the effect of two common intestinal protozoa (*Blastocystis* and *D. fragilis*) on psychological symptom severity. The majority of research into the BGMA focuses exclusively on the bacterial component of the GM, with little to no attention given to other members of the GM such as protozoa. *Blastocystis* and *D.*

fragilis specifically were important to consider given the ongoing controversy over their role in human health and disease (e.g., Garcia, 2016; Lepczyńska et al., 2017). It was also important to assess the impact of protozoa, as there is the potential that their presence or absence confound conclusions regarding the role of gut bacteria on symptom expression. The findings of Paper 2 demonstrated that;

- The symptom severity of those who carried *Blastocystis* and/or *D. fragilis* did not significantly differ to those who had tested negative for intestinal protozoa.
- While this study adds weight to the evidence suggesting that these protozoa may not be harmful, its main purpose was to act as a first step for the consideration of non-bacterial microorganisms that also make up the BGMA in research going forward.

In the context of the current thesis, the findings of Paper 2 served to inform the inclusion/exclusion criteria for Paper 3. As it was found that neither protozoan effected psychological symptom expression, those who had tested positive to *Blastocystis*, *D. fragilis*, or both were retained for subsequent analyses.

Finally, Paper 3 aimed to investigate the relationship between GM at the species level and symptom severity across four psychological symptom domains (Depressive, Neurocognitive, Stress and Anxiety, and Sleep and Fatigue) between males and females. The benefit of species level exploration led to a more precise understanding of the interaction between specific microbes and symptom expression, which had been lacking to date. The findings of Paper 3 demonstrated that;

- Several GM species were associated with psychological symptom severity.
- These associations clearly differed between males and females, providing support for the concept of the microgenderome.
- These findings add weight to a growing body of evidence which suggests that GM are associated with psychological symptom expression, but also reflect the inconsistencies within the literature in regards to these associations.

While further research is needed, these findings point towards a paradigm shift in psychology away from a CNS-centric focus towards a more holistic conceptualisation of psychological health and disorder.

7.2 Parallels Between Psychology and Microbiology

Social science research has always aimed to measure the ‘immeasurable’. That is, abstract concepts that are not tangible and cannot always be directly observed. Yet, social science research has provided a good understanding of what these concepts are, how they impact a person’s daily

functioning, and what other aspects of a person's life they are related to. This is one of the distinguishing factors between the social sciences (such as psychology) and the natural or bench sciences (such as microbiology), where what is being measured is tangible, directly observable, and relatively easily quantifiable. However, the sheer complexity of the GM, and the immense intra- and interpersonal differences in GM composition (Lloyd-Price et al., 2016; Turnbaugh et al., 2009) has introduced a challenge not typically faced in the natural sciences, but one that is familiar to the social sciences. This is demonstrated by several factors such as the inability to precisely define a "healthy" GM composition (Rinninella et al., 2019), varying ways of collecting samples (Tang et al., 2020), and several different ways through which samples can be analysed and quantified (Bharti & Grimm, 2021). Therefore, approaching an investigation of the BGMA from a predominately psychological perspective may offer a benefit to microbiology in that the principles and practices more commonly used in social science research may help to make sense of the GM, which resembles the complexity and abstract nature of psychological phenomena.

In psychology, it is well established that a number of factors contribute to a client's presenting problem. This is evidenced by the multidisciplinary biopsychosocial model that proposes an individual's biological, psychological, and socio-environmental factors interact in a unique way that results in their presenting problem (Campbell & Rohrbaugh, 2006; Engel, 1977). While two people may present with the same disorder (e.g., depression), they may have a distinctly different combination of biological, psychological, and social factors which coalesced to result in their disorder. The individual experience of a particular disorder may also be markedly different between two individuals. This makes psychological phenomena highly complex and individualistic. Therefore, in practice, a psychologist must gather a range of information pertaining to an individual's past and current circumstances to understand their current psychological state. This is done through a clinical interview based on the Four P model of case formulation (Predisposing, Precipitating, Perpetuating, and Protective). In formulating an individual's presenting problem, a psychologist takes an individual and targeted approach to each client's unique circumstances. In accordance with the biopsychosocial model, psychologists may ask questions regarding a client's biology. These questions generally refer to genetic predisposition (a family history of psychological disorders), medical illnesses, and drug and alcohol use. Paper 1 presented a review of the BGMA literature in a way that demonstrates how GM may be aligned with each of these four P's. This serves to demonstrate that consideration of GM, to varying degrees, should be integrated into a client's formulation. It is not suggested that psychologists refer every client for a faecal microbiota analysis, however, this may sometimes be appropriate. For example, being aware that several factors impact on GM composition, psychologists may incorporate questions about a client's diet or recent dietary

changes. Psychologists may consider referring a client for an FMA if social and emotional factors are not indicated.

This complexity of psychological phenomena is paralleled in microbiological research. It has been established that there are a number of factors which can alter the composition of a person's GM that may implicate bottom-up, top-down, or multidirectional processes. These alterations can range from short term (<24 hours; David et al., 2014; Wu et al., 2011) to long term, or potentially life long (Conlon & Bird, 2014; Roubaud-Baudron et al., 2019). Influential factors including but not limited to birth mode, feeding method, diet, exercise, smoking, antibiotic use, probiotic and prebiotic use, could be considered as bottom-up. Top-down processes that alter GM composition include a person's psychological state (Madison & Kiecolt-Glaser, 2019). Acknowledgement of multidirectional processes accounts for the influence of the BGMA on psychological symptoms via the GM's effect on other organ systems such as the liver (Schwenger et al., 2019) and the heart (Novakovic et al., 2020). Multidirectionality therefore pays respect to the immense complexity of the interactions between GM and other bodily systems, and their impact on an individual's psychological wellbeing (De Hert et al., 2018; Polis & Fernandez, 2015).

In the papers presented within this thesis, state-based symptom severity (over the last 7 days) was associated with concurrent GM composition, as responses to the BPQ were provided within temporal proximity of the collection of the patient's stool sample. Therefore, the GM was also considered to be state-based. While in psychology, the distinction between state and trait-based factors is reasonably well understood, it remains difficult to completely disentangle the influence of state and trait on an individual's current behaviours and psychological experiences. For example, determining the extent to which an individual's anxiety is due to a current stressor (state) cannot be considered completely independently of their personality (trait), as someone who has high neuroticism would be predisposed to anxiety (Vinograd et al., 2020; Wauthia et al., 2019). So too, it is difficult to ascertain whether an individual's behaviour in their interpersonal relationships is due to current circumstances or due to their attachment style, established in early childhood (Hazan & Shaver, 1987; Simpson & Rholes, 2017). This difficulty in clearly distinguishing state and trait is mirrored in regard to GM and their association with symptom expression. For example, most research demonstrates the associations between current GM composition and current symptom expression. This is because it is difficult to retrospectively sample an individual's early GM composition. However, since it is believed that early colonisation and dysbiosis can influence health later in life (e.g., Sarkar et al., 2021), this is better conceptualised in terms of a trait. Early colonisation of the gut plays an important role in the development of the CNS, ANS, and HPA, as well as the development and entrainment of the immune system (e.g., Belkaid & Hand, 2014; Carabotti

et al., 2015; Vagnerová et al., 2019; Zheng et al., 2020). Therefore, even if dysbiosis is ameliorated, such early effects of GM may underlie any current symptom expression, regardless of current GM composition.

As such, any attempt to clarify the distinction between state and trait based influence in the BGMA will be difficult to disentangle. One reason is that sampling techniques of the GM vary between studies. For example, culture-based methods such as the one used in the current thesis counts only viable (live) cells, whereas sequencing techniques based on DNA cannot distinguish between live and dead cells (Cangelosi & Meschke, 2014). Another important question that has received little attention is whether dead cells can still have an impact on host health. The majority of research on this particular topic is in regards to the physiological functions of live probiotic species compared to the same heat killed species, but does demonstrate that non-viable (dead) bacteria may still influence host health. For example, Sugahara et al. (2017) found that both live and heat-killed *Bifidobacterium breve* demonstrated immune-modulating effects, however live *B. breve* demonstrated a greater effect on intestinal metabolism. Reviews by Adams (2010), Maehata et al. (2021), and Piqué et al. (2019) conclude that dead cells still have the potential to modify biological responses. Therefore, the lines between trait and state remain blurred. While still difficult to entirely separate, social science research has made great strides in unpacking the influence of state versus trait. Through ongoing and rigorous research, assessment practices have improved over time which has allowed for measures with greater psychometric properties to be developed. Using personality as an example, early measurements of personality came in the form of projective tests such as Rorschach's inkblot test and the Thematic Apperception Test. These measures are highly subjective, meaning they have inherently poor psychometric properties. As a result of continued scientific research, today, there are several measures of personality that demonstrate strong psychometric properties such as the NEO Personality Inventory (McCrae et al., 2010; Young & Schinka, 2001). Over time, and through scientific research, consensus develops regarding concepts and the way to measure these concepts. Through this consensus, models are developed which allow for a better, more complete understanding of a particular phenomenon (such as models of personality). BGMA research is still in a relatively early stage, and further research is needed to develop better assessment practices which will allow for a greater understanding of the associations between GM and symptom expression.

The cognitive revolution in psychology during the 1950s marked a shift away from behaviourism which was the dominant paradigm in psychology at the time. The need for multidisciplinary collaboration to solving problems was becoming clear, leading to the redefining of psychology among other disciplines such as anthropology, linguistics, and neuroscience (Miller,

2003). Today, it is increasingly being recognised that the discipline of psychology could again benefit from another redefinition, considering psychological phenomena in the context of microbiological influences (e.g., Allen et al., 2017). This is not to suggest that shifts in the discipline of psychology are dictated by other disciplines, but instead, that it must evolve in light of evidence which suggests new or additional ways in which the human experience can be best conceptualised and explored.

The cognitive revolution gave rise to models which explained various psychological or cognitive processes. For example, models of attention (e.g., Broadbent, 1957) and memory (Atkinson & Shiffrin, 1968). Knowledge of the brain structures associated with particular functions typically came from reports of individual cases where specific structures had been damaged or removed. For example, the case of Henry Molaison who had the majority of his medial temporal lobes removed, including the hippocampus and most of the amygdala, demonstrated the role that these structures play in memory functions. Additionally, the case of Phineas Gage demonstrated the role of the frontal lobe in personality.

While undeniably valuable, these models described and explained cognitive processes in a modular and piecemeal fashion. Newell (1973, 1990) argued that an integrative theory of cognitive psychology was lacking. In the mid -1990s, Mapou (1995) proposed a framework for cognitive assessment which suggested an integration of multiple cognitive skills and processes. This framework was hierarchical, suggesting that higher order skills (such as learning and memory) could only be achieved if a person demonstrated functional capacity of lower order skills (such as general intellectual, attention, reasoning, and language abilities). The literature on executive functioning also demonstrates that multiple interrelated cognitive systems are involved in performing complex cognitive tasks essential for mental and physical health, and social functioning (Diamond, 2013; Lezak et al., 2004).

The current state of BGMA research is therefore analogous to the early stages of the cognitive revolution, where separate systems were conceptualised in a modular fashion. Much of the literature focuses on associations between single species, genera, or phyla and specific symptom expressions or disorders. This does not respect the complexity of the GM and the communication between the members of this diverse ecosystem which supports symbiosis and homeostasis, or drives dysbiosis (e.g., Bauer et al, 2018; Ghoul & Mitri, 2016). This is exemplified by the results presented in Paper 2. There are two perspectives from which the results of Paper 2 could be interpreted. The first is that protozoa *Blastocystis* and *D. fragilis* are non-consequential to psychological symptom expression and are therefore not of concern. As such, individuals who test positive for these protozoa do not need to be excluded from further analyses. For the purposes of

the current thesis, and in consideration of the limitations posed by the retrospective data, this was the perspective taken. This interpretation is a practical one given the nature of microbiota data. For instance, out of the overall sample in Paper 2 who had been tested for intestinal protozoa (*N*= 979), 58% tested negative to any protozoan. This was exacerbated when separating for sex and by protozoan (e.g., *Blastocystis*, *D. fragilis*, *or co-carriage*). Even with 258 male participants, there was an insufficient sample of males who carried *D. fragilis* or co-carried *Blastocystis* and *D. fragilis* to allow for any meaningful statistical analysis. This is before controlling for the myriad of other factors which could potentially influence the action of these intestinal protozoa such as use of medications, age, diet, duration of carriage of protozoan, and so on, notwithstanding the limitations of the data (e.g., Leeming et al., 2019; Lukeš et al., 2015; Rinninella et al., 2019; Vich Vila et al., 2020).

An alternative perspective is that the influence of *Blastocystis* or *D. fragilis* on psychological symptom expression cannot fully be determined in isolation of additional information, such as an individual's bacterial or fungal composition. It may be that symptom expression is dependent on particular interactions between specific protozoa, bacteria, and fungi. This is reminiscent of Mapou's (1995) framework of cognitive assessment and the concept of executive functioning which acknowledges an interdependence of multiple systems in performing a particular task. Giving weight to this perspective is the fact that the role of *Blastocystis* and *D. fragilis* in health remains controversial, with widely disparate research findings (Garcia, 2016; Lepczyńska et al., 2017). It is probable that these inconsistencies are, at least to some extent, due to unmeasured interactions between these protozoa and the other constituents of an individual's GM. These unmeasured interactions may also explain the relatively small effect sizes uncovered in the current thesis. While Paper 2 included a brief analysis of the moderating effect of Bacteroidetes and Firmicutes (at the phylum level) demonstrating no interaction with *Blastocystis* and/or *D. fragilis*, it is likely that interactions would occur at lower taxonomic ranks such as species. Investigation of species level interactions was beyond the scope of Paper 2 and this thesis, however, this should be considered as an important future direction for all BGMA research. As the majority of BGMA research to date has focused on the bacterial component of the gut, much less is known about the potential influence of protozoa and their interactions with other members of the GM (Coyte & Rakoff-Nahoum, 2019; Shkoporov & Hill, 2019). Shifting towards a broader investigation of the interaction between bacteria and other members of the GM is in line with the concept of the holobiont. This suggests that to understand the relationship between GM and symptom expression, the interaction between all genetic material must be considered.

The results of Paper 3 also demonstrate the need for this change in the direction of BGMA research. In reviewing previous literature, inconsistencies in the associations between a single

species or genus with symptom expression was evident (e.g., Aizawa et al., 2016; Lai et al., 2021; Nguyen et al., 2019; Shen et al., 2018). Additionally, the findings presented in Paper 3 contribute to these inconsistences, demonstrating that species commonly used in probiotic supplements such as *Lactobacillus plantarum, L. delbruckii, L. acidophilus,* and *L. paracasei* were found to be positively associated with symptom severity across multiple psychological symptom domains. This demonstrates that within the current sample, there was (were) some underlying factor(s) that resulted in these positive associations with symptom severity. It is not unreasonable to suggest that the outcome of increased symptom expression could be the result of inter-species interactions. Current knowledge of precise interactions between members of the GM remains limited (Coyte & Rakoff-Nahoum, 2019) and future research must investigate the interaction between multiple species, rather than focusing on any single microorganism to fully appreciate the role of GM on symptom expression.

Further, the results of Paper 3 also clearly demonstrate that the microgenderome is a valid concept, adding weight to a growing body of literature (e.g., Chen et al., 2018; Wallis et al., 2018; Vemuri et al., 2019). These findings demonstrate that as well as being considered in the presence of other microorganisms, investigations of microbe-host relationships must also take into account host characteristics such as an individual's balance of sex-specific hormones. A promising aspect of BGMA research is the prospect of moving towards a more personalised approach to diagnosing and treating psychological disorders. If this is the ultimate goal for the application of BGMA in psychological research, the research itself must reflect the individual differences that influence microbe-host relationships.

7.3 Reconceptualising Who We Are

BGMA research over the past decade or so has demonstrated that a CNS-centric approach to conceptualising psychological symptoms and disorders is antiquated. While a CNS-centric approach has undoubtedly contributed to our current understanding of psychological disorders, it is clear that there is so much that is yet to be uncovered. The CNS resides, and can only function within the body. As such, it seems illogical to ignore the other aspects of the human body that are essential to its functioning. Through the functions that they perform, the GM have been demonstrated to be essential for human health (e.g., Sekirov et al., 2010; Valdes et al., 2018). Investigation of the GM has potential to revolutionise the way that psychological disorders are conceptualised and managed. Prevalence rates of depression and anxiety continue to be so high that they are referred to as common mental disorders (WHO, 2017), occurring in approximately 4.4% of the global population, but often reported at higher rates (e.g., Bandelow & Michaelis, 2015; Kalin, 2020; Lim et al., 2018; OECD, 2021). While there are several pharmacological and psychological therapies available, rates of

resistance to treatment are high (Howes et al., 2021). Additionally, the aetiology of these disorders is yet to be fully elucidated. The discipline of psychology specifically aims to understand how people think, feel, and behave. It is essentially concerned with understanding the human experience. In doing so, the human being is considered distinct and separate from other life forms. On the surface human beings present as a complex entity, however this conceptualisation belies the interdependence between humans and the organisms with which they coexist. The concept of the holobiont refers to a single biological entity that is comprised of a host and its associated non-human cells (Salvucci, 2019; Simon et al., 2019; Theis et al., 2016; van de Guchte et al., 2018). This is a concept that is far more familiar to biologists than it is to psychologists. However, this thesis suggests that this must be a concept that psychologists become familiarised with. Given that there is an increasing weight of evidence suggesting that GM play a role in the development and function of the CNS and HPA axis (e.g., Vagnerová et al., 2019; Wang et al., 2018), and are associated with psychological disorders (e.g., Jiang et al., 2015; Jiang et al., 2018; Lai et al., 2021; Nguyen et al., 2019; Strati et al., 2017; Vogt et al., 2017; Zheng et al., 2016), this is highly relevant to our understanding of structures and concepts that have always been the focus of psychologists. To not consider an individual as a holobiont, including an individualised GM, is ignoring an integral part of their biology, and therefore restricting our understanding of the human experience. This notion of the identity of a human being intertwined and inseparable from its microbial constituents is reflected by several authors (e.g., Dethlefsen et al., 2007; Gligorov et al., 2013; Hutter et al., 2015). The notion of the holobiont is also supported by the co-evolution of humans with their resident GM (e.g., Lloyd-Price et al., 2016; Ursell et al., 2012). When considering the interpersonal differences which make it impossible to establish a 'healthy' GM, and that an individual's GM is as unique as a fingerprint, this notion of personal identity has some weight. This idea that an individual's GM forms part of their personal identity is an interesting one, which brings with it numerous ethical considerations (Rhodes, 2016). Before even considering the associations between GM and psychological symptoms and disorders, from a philosophical perspective, this field of research (the BGMA), challenges the current conceptualisation of what it is to be human. Given that psychologists specifically deal in the business of the human experience, consideration of the BGMA is indispensable.

Cartesian dualism, the philosophy that the mind and body are separate entities, is likely to have contributed, at least to some extent, to the separation of psychology and biology. The conceptualisation of the biopsychosocial model by Engel in 1977 demonstrated the importance of considering biological (and social) factors when trying to understand psychological phenomena. This model progressed understanding of psychological conditions through its multidisciplinary and more holistic approach. Research into the BGMA fits into the framework of the biopsychosocial model, but

suggests that rather than looking at a person's biology at the macro level (general health status, family history, substance use), the micro level also has a wealth of information to provide in regards to understanding predisposing, precipitating, and perpetuating risk factors. Likewise, the understanding of the protective nature of a person's microbiology has the potential to inform possible auxiliary treatment options. Currently, psychotherapy and psychopharmacology (or a combination of the two) are the predominant arms of psychological treatment. GM modulation is an emerging auxiliary treatment option, either on its own or in conjunction with current treatments (Butler et al., 2019; Meyyappen et al., 2020). The addition of GM modulation to an individual's treatment plan may be particularly useful in cases where clients display treatment resistance to typical psychopharmacological interventions. This is due to GM being able to alter the bioavailability, bioactivity, or toxicity of a drug (Walsh et al., 2018; Weersma et al., 2020). Treatment resistance to psychopharmacological interventions for anxiety and depression are considered to be approximately 30% (Bystritsky, 2006; Jaffe et al., 2019; Ansara, 2020). The mediating effects of GM on medication action may also provide insight into the side effects experienced by some clients as a result of taking these treatments. These side effects may themselves be a barrier to treatment compliance (Fortney et al., 2011; Ho et al., 2017). As such, using GM modulation as an additional treatment option may alleviate certain side effects. However, further research investigating the interaction between GM and psychopharmacological treatments is critical.

7.4 Limitations

Study specific limitations have been outlined in the discussion sections of Paper 2 and Paper 3. Here, overarching limitations will be discussed as they relate to BGMA research in general and in reference to suggestions for future research. Some of the limitations discussed are not unique to the current thesis, but are a common issue in BGMA research generally which warrant consideration.

7.4.1 Retrospective Data

The current thesis was only possible owing to the availability of Bioscreen's clinical dataset. The retrospective nature of the data allowed for the collection of an impressively large number of participants (N=9812) and microbial variables (>500). Data of this magnitude was thought to present a unique opportunity for complex statistical modelling, however in analysing the data, it became apparent that there were a number of unanticipated issues that emerged. The first issue related to the retrospective nature of the data collection. Specifically, the data were not originally collected for research purposes. Data were collected as part of the investigatory process for intestinal dysbiosis by clinicians. As such, microbial counts were the only variables of interest. However, for research purposes, the measurement of confounding variables is important, particularly in the case of BGMA research where there are a multitude of factors that can influence the composition of an individual's

GM. The dataset used for the current thesis did not include potentially important information regarding several confounding variables such as an individual's current diagnostic status, dietary information, medication use, antibiotic or probiotic use, and a variety of other lifestyle factors.

Another issue related to the magnitude of the diversity in the dataset. As psychologists we could not envisage that there would be so few cases that would share a GM composition to allow for cluster analysis, or other pattern identification in the sample. Although our team comprised both psychologists and microbiologists, this thesis lies at the intersection of both disciplines, introducing unique challenges that demanded a reappraisal of the initial research design. Ultimately, the cost of these limitations restricted the use of more powerful modelling and required an exploratory approach to analysis.

To date, it remains unclear whether changes in GM lead to psychological symptom expression or disorder, or whether psychological conditions precede GM changes. Available evidence suggests that this relationship is bidirectional, and that both sequences are possible (Winter et al., 2018). For example, GM are thought to influence psychological functioning through complex interrelated processes involving neuronal, endocrine, immune signalling, and metabolism (Clapp et al., 2017; Cox & Weiner, 2018). On the other hand, stress and activation of the HPA axis can influence intestinal permeability which can alter GM composition (Farzi et al., 2018). As such, it is likely that complex interactions between GM, disease states, and varying symptom expressions exist. These interactions are further complicated when considering the influence of other behavioural factors associated with illness (e.g., dietary changes and medication use) which can themselves alter GM composition (Leeming et al., 2019; Vich Vila et al., 2018). Having information regarding these factors would allow for more detailed analysis of the associations between GM and symptom expression, which could provide further clarity around whether symptoms are preceded by GM composition or vice versa.

Given that participant's current diagnostic status was not included in the dataset, the inclusion of a 'healthy' control group was precluded. So too was the demarcation of different clinical groups. While not essential when assessing associations between variables, determining whether associations between GM and symptom expression differed between healthy controls and diagnostic groups could provide further insight into these relationships. As such, the results presented within Paper 3 in particular, should be interpreted as associative only. While Paper 2 compared the self-reported psychological symptom severity of those who tested positive for *Blastocystis*, *D. fragilis*, or co-carriage to those who tested negative for intestinal protozoa, not

knowing the diagnostic status of those who tested negative to intestinal protozoa still precludes the ability to label these participants as a control group.

A further limitation relating to the retrospective nature of the dataset, and particularly that the data was collected over a period of 2 and a half years, is that the MALDI-TOF databases are updated when new species are identified (Singhal et al., 2015). While this would generally be considered ideal (especially in clinical practice), as the most up-to-date database allows for greater identification of species when available, it adds to the heterogeneity of the overall sample over an extended period of time. For example, if a particular species was added to the database in 2014, sample analyses prior to its addition would not have identified that species and it would instead present as missing data, or would have an alternative classification (such as "*Bifidobacterium* species unidentified").

While a limitation of the current thesis, retrospective designs may still offer great value in future BGMA research. Retrospective designs are useful in instances where the number of individuals who fulfil a particular condition are rare (Talari & Goyal, 2020). This was clearly exhibited in the current thesis where a large number of microbes could not be analysed due to an insufficient number of participants who had those particular microbes. However, if retrospective datasets are to be used, they must be designed from a research perspective. When constructing databases, clinicians must be cognisant to include as many confounding variables as feasibly possible (including, but not limited to diagnostic status, probiotic and/or antibiotic use, dietary factors). Additionally, the homogeneity of data collection needs to be prioritised with protocols in place to minimise, or to at least identify possible issues of heterogeneity.

7.4.2 Missing Data

Missing data in BGMA research is inevitable. While over 2000 species of GM have been identified (Almeida et al., 2019), a healthy human GM is thought to be comprised of between 100 and 500 species (King et al., 2019; Quigley, 2013). The immense interindividual diversity of GM composition means that it is extremely unlikely for any two people to have the same composition. Therefore, it is not unreasonable to suggest that large amounts of missing data are the rule rather than the exception. However, in all the studies reviewed for the papers presented within this thesis, there was no explanation about how missing data was handled. This is despite calls for reporting of missingness, and how missingness is treated to ensure that readers can determine the robustness and validity of research findings (Karahalios et al., 2012; Lee et al., 2021; Vandenbroucke et al., 2007). Given that this information is currently unavailable, efforts were made within the current thesis to retain as much valid data as possible, without the use of multiple imputation which is not considered appropriate for use with microbial data (Kaul et al., 2017) or when missingness exceeds

40% (Jakobsen et al., 2017) which was the case within the current dataset. Therefore, listwise deletion was utilised in Paper 2, while pairwise deletion was employed in Paper 3. In retaining as much viable data as possible, the goal was to allow for the most meaningful data analysis. The difference in technique used between Paper 2 and Paper 3 was due to the nature of the data being assessed. For example, in Paper 3 where the association between 152 different microbial species and psychological symptom severity was being assessed, the use of listwise deletion would have resulted in zero valid cases. It is important for BGMA researchers going forward to identify and outline specific methods for dealing with missing data. This would allow for consistent application of any specified method across studies.

Missing microbial data in BGMA research cannot be treated in the same way as typical missing data in social science research, as the missingness itself is due to an individual's underlying biology (Kaul et al., 2017). An additional reason that this type of data cannot be treated in the same way, is that the absence of presence does not necessarily mean the presence of absence. For example, if a microorganism was present within a stool sample, but its abundance was below detectable limits (as detailed in Appendix C), it would have appeared as an empty cell (missing) in the retrospective dataset, when in fact it was present in the hundreds of thousands. The complication is that it is also possible for microorganisms to be completely absent. In the current dataset, these instances could not be distinguished from cases where microbes were present, but below detectable limits. This made it extremely difficult to deal with missingness and limited the viable options for analysis. Kaul et al. (2017) propose that cases where microbes are in fact not present should not be treated as missing data, but instead refers to these cases as 'structural zeros'. It is essential that going forward, efforts are made to address this issue by working towards a feasible solution to treating missing data. Statements regarding the proportion of missingness and how it is treated must be included in future BGMA research. This challenge of the 'structural zero' can be reduced by using methods that seek to capture all microorganisms, for example 16S rRNA gene sequencing.

7.4.3 Sampling and Quantifying Microbiota

Stool sampling in human studies is the most established and widely used procedure of measuring GM (Al Bander et al., 2020). Although, there is evidence to show that stool samples provide a weaker reflection of dysbiosis compared to mucosal tissue samples in new-onset Crohn's disease patients (Gervers et al., 2014). As such, faecal samples are not fully representative of overall GM composition, as they do not include mucosa-associated microbes (Huse et al., 2014), or those that do not reside in the colon (i.e. microbes residing in the small intestine; Hillman et al., 2017; Leite et al., 2020). While mucosal tissue samples may provide a more representative snapshot of GM, this

method is far more intrusive, making it less suitable for healthy participants in research (Tang et al., 2020), and also comes with its own set of limitations, making it far less commonly used in research (Al Bander et al., 2020). Technological advances, such as the development of the smart capsule, which is able to collect microbial samples along the GI tract in a non-invasive manner, will assist in providing a more complete representation of GM (Tang et al., 2020; Waimin et al., 2020).

There is considerable conflict surrounding the best method to quantify GM that must be understood when reviewing microbiology literature. The development of approaches to culture bacteria, conceptualised as culturomics (Lagier et al., 2012), is what Lagier et al. (2018) describe as "the rebirth of culture techniques in microbiology" (p. 540). Greub (2012) defined culturomics as a method of describing microbial composition through high-throughput culture which was made possible by MALDI TOF mass spectrometry. MALDI TOF mass spectrometry has been described as a popular and powerful tool due to its cost-effective, rapid, and precise identification of genus and species level data (Hou et al., 2019; Samb-Be et al., 2014; Singhal et al., 2015; Strejcek et al., 2018).

However, others assert that culture-based methods in microbiology, such as were used in this study, should be considered as obsolete (Al-Awadhi et al., 2013) and are widely criticised for their inability to detect 'unculturable' microorganisms (e.g., Almeida et al., 2019; Lau et al., 2016; Tang et al., 2020). However, Lagier et al. (2015) dispute the claim that certain microorganisms are 'unculturable' as a misnomer, arguing that all microorganisms are able to be cultured once the proper tools/conditions for culturing have been found. However, it must also be noted that when using culture-based methods there may be an investigation bias, therefore what is found is restricted to the medium adopted by the microbiologist, even though culture-based methods allow for the detection of less abundant microorganisms relative to non-culture methods.

The introduction of culture-independent sequencing such as 16S rRNA gene sequencing and shotgun metagenomic sequencing have undoubtedly highlighted the diversity of GM and the large number of previously uncultured gut bacteria. These techniques also allow for the functional analysis of the GM (Nichols et al., 2018). However, such culture independent techniques still have a number of limitations. Culture-independent techniques are susceptible to depth bias which refers to their inability to detect low-abundance organisms (e.g., Hiergeist et al., 2015; Lagier et al., 2015). Lagier et al. (2012) suggest that metagenomic methods are unable to detect bacteria present in low concentrations. An additional limitation of culture-independent metagenomic techniques is viability bias, which refers to their inability to *distinguish* between live bacteria and transient DNA of dead microbes (Emerson et al., 2017). Using 16S rRNA techniques also limits the ability to identify microbes at the species and strain level due to its poor resolution (Cheng et al., 2019). Moreover,

this technique limits the ability to detect non-bacterial members of the GM which do not have the 16S rRNA gene (Peterson et al., 2021).

More recently, shotgun metagenomic sequencing has addressed some of the limitations of 16S sequencing. Shotgun metagenomic sequencing substantially increases resolution and is therefore more sensitive and accurate, allowing for identification of all types of organisms, including viruses, fungi, and protozoa present at lower abundances (Brumfield et al., 2020; Donovan et al., 2018; Lokmer et al., 2019; Peterson et al., 2021; Wylezich et al., 2019). While shotgun metagenomics also offers identification at the species and strain level of data (as opposed to culture-dependent methods identification of genus and species level data), shotgun sequencing comes at a relatively high monetary cost which is a barrier to its large-scale use (Rausch et al., 2019). Others have suggested that the best approach would be using culture-dependent alongside molecular techniques, where the advantages of each together could outweigh the disadvantages of a single technique alone (e.g., Al-Awadhi et al., 2013; Fenske et al., 2020; Zampieri et al., 2021). While this may not be practically feasible, the adoption of a method of quantifying and sampling the GM is dependent on the theoretical position of the researcher regarding the importance of (for example) the presence of non-bacterial organisms, live versus dead microorganisms, and taxonomic specificity.

7.4.4 Symptom Measurement

Psychological symptoms were measured using the BPQ, a symptom checklist developed by Bioscreen. In order to validate the use the BPQ in the studies presented within this thesis, the questionnaire was subjected to statistical analysis to determine underlying factors (results of which were presented in online resource 1 of Paper 2). As such, the BPQ is not a well validated tool to measure psychological symptoms. However, in unpublished work undertaken by the broader research group, the BPQ demonstrated good convergent validity with scales such as the DASS-21, CFQ, and PSQI using very similar factor structures to those in the current thesis. Given the retrospective nature of the dataset, it was not possible to use alternate measures of psychological symptom expression, and it was not possible to test convergent or divergent validity due to the inability to add other measures. Given the methodological heterogeneity with regards to the microbiological component of BGMA research, future studies should ideally use well-validated measures of psychological symptoms so that such outcomes are measured consistently.

7.5 Suggestions for Future Research

Social sciences such as psychology have centred around making sense of complex phenomena by using small, targeted studies. This differs to the direction of microbiome research and its pursuit of big data (e.g., Cheng et al., 2019; Cullen et al., 2020; Jiang & Hu, 2016; Sung et al.,

2016; Zha et al., 2021). As a result of the immense intra- and interpersonal differences in GM, the larger the sample, the greater the disparity and heterogeneity of a sample, as demonstrated within the dataset used in the current thesis. As such, current methodological approaches to investigating GM is creating an inherent flaw in BGMA research. Applying a similar approach taken in psychological research to BGMA research, or microbiology research in general, may help to solve some of the issues that exist within BGMA research. The cost-benefit ratio of large datasets is the pursuit of greater power, but with likely increased heterogeneity. Thus, it is suggested that future research should trade sample size for more meaningful groups with predetermined sample characteristics. Going forward, longitudinal and repeated measures studies are likely to provide a greater precision of information as opposed to larger population-based cross-sectional studies. They will allow for changes in microbial composition and the symptom expression of an individual to be tracked over a period of time. Given the immense interpersonal differences in GM composition, repeated measures designs may be better suited to exploring associations between GM composition and symptom expression, as such designs remove individual differences/error. Repeated measures designs have been implemented in studies investigating the efficacy of probiotics on symptom expression (e.g., Kazemi et al., 2019; Lew et al., 2019; Tillisch et al., 2013; Wallis et al., 2018). Additionally, repeated measures designs may also be valuable when no intervention is administered, but instead to track the relationship between GM and symptoms over time.

In the early days of neuropsychological research, knowledge was developed from case studies of disordered functioning (Henry Molaison, Phineas Gage), however the study of healthy or neurotypical individuals allowed for much greater understanding of neuropsychological processes. Comparisons between healthy and non-healthy individuals across time will allow for a greater insight into the specific associations between GM and the 4 P's of psychological case formulation. Therefore, going forward, it is important that both healthy and non-healthy individuals (ranging from those with specific diagnoses to those suffering from sub-clinical symptoms) are included in BGMA research. Individual's specific diagnoses should be recorded as it may be important to consider those in different diagnostic groups independently of one another. The magnitude of this importance remains unclear. Tracking both healthy and non-healthy individuals over time, while considering different diagnostic groups separately will also provide insight into the direction of the relationship (which precedes which) between GM composition and symptom expression. The inclusion of healthy individuals in any analysis also provides a control group which enhances the scientific rigor of research (Kinser & Robins, 2013). This will facilitate a shift from observational to experimental design.

While it is always important to well-plan any prospective study, it is especially important with regards to BGMA research. This is due to the multitude of factors that could be considered confounding variables, which should be carefully considered and measured. Well-designed longitudinal repeated measures studies will allow for greater investigation of the potential mechanisms which underly the associations between GM and symptom expression. To date, the precise mechanisms remain elusive and difficult to study. Greater understanding of these mechanisms will facilitate the application of BGMA research in psychological practice. In doing so, future research must focus on species or strain level data. As technology improves allowing for identification at these more specific taxonomic ranks, the specificity of this information provides more value than investigation at broader taxonomic ranks. Moreover, it is suggested that future BGMA research move away from focusing solely on compositional assessments of GM, but to also include functional analyses which also involve microbial metabolites. Taken together, this information will undoubtably provide more practical and clinically useful information than is currently available.

Paper 3 adds to a growing body of literature supporting the concept of the microgenderome. However, as with many studies to date, evidence for this concept is based on divergent associations between male and female participants (e.g., Chen et al., 2018; Taylor et al., 2019; Wallis et al., 2016). To further understanding regarding the relationship between hormonal levels and GM composition, it is recommended that where possible, future studies include biomarkers such as individual oestrogen/testosterone levels. The inclusion of additional biomarkers as objective measures (e.g., cortisol levels, immunoglobulin levels) would provide researchers with greater control, and would allow for a more accurate understanding of the complex multi-directional relationships between host systems and GM.

7.6 Concluding Remarks

Advances in knowledge regarding the connection between the brain and GM shows outstanding promise in revolutionising the way that psychological illness, and its prevention and treatment are conceptualised. It is now clearly established that there is an association between various gut microbes and psychological symptom expression. This paradigm shift away from the current CNS-centric approach of the discipline of psychology is in line with the concept of the holobiont which considers the human host and its resident GM to be a single superorganism. Not giving credence to the essential role of GM in contributing to what it is to be human would be to limit the advancement of our understanding of the human experience, which includes psychological phenomena. The physiological structure of the brain, the CNS, and PNS are taught in undergraduate psychology degrees to provide an understanding of the biological aspects of behaviour. However,

information regarding the essential role of other physiological factors, such as GM, is currently lacking. This is not to say that a significant proportion of a psychology degree should now be focused on microbiology or physiology, however, the significant role of GM in human development and behaviour is crucial to a complete understanding of psychological phenomena. Given the evidence to date, curriculum should be updated to reflect current knowledge.

The GM presents an opportunity for psychologists, as part of multidisciplinary teams including microbiologists, to have a greater understanding of the aetiology of psychological disorders. This will inevitably inform more efficacious treatment options for clients, particularly those who may currently be treatment resistant. This is also in line with the increasing interest in personalised medicine, which aims to improve treatment outcomes and reduce adverse events for clients (Cutter & Liu, 2012). Given the vast interpersonal differences in GM composition, treatments which target the GM are, in essence, the most personalised.

There is an immense interest in GM which has resulted in great excitement about modulation of GM as an additional avenue of treatment in psychological practice, and a call for consideration of gut health in psychological functioning is the central tenet of this thesis. However, there is still a long way to go before GM modulation can be routinely considered for use as an adjunct treatment for psychological symptom expression and disorder. To date, knowledge regarding precise mechanisms of action is lacking. BGMA research is also hindered by several methodological and practical limitations as outlined above. Additionally, the majority of research, particularly in humans, is associative. Together with advances in technology (such as the development of smart capsules for measuring GM more completely), continued multidisciplinary research will advance knowledge regarding the associations between GM and psychological symptom expression. Further, given the abundance of evidence demonstrating these associations, the goal of BGMA research must now shift to gaining a clearer mechanistic understanding of these relationships. This will be facilitated by continued collaboration between researchers from microbiology and psychology disciplines. Methods of inquiry used in psychological research will be of great benefit to furthering understanding of the BGMA.

The discipline of psychology is on the cusp of a paradigm shift which could revolutionise current conceptualisations of psychological health and disease. While theoretically exciting, the potential practical implications for psychologists and their clients are equally important and exhilarating. While there are a number of challenges to be overcome in order to advance our current knowledge regarding the complex multidirectional relationships that make up and facilitate the BGMA, continued research with consideration of the aforementioned limitations and suggestions for

future research is of utmost importance. While this may seem a daunting task, technological advances, along with multidisciplinary teams sharing research practices, will make what is not possible today a reality of the future.

References

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science translational medicine*, *6*(237), 237ra65. https://doi.org/10.1126/scitranslmed.3008599

Aaronson, S. T., Carpenter, L. L., Conway, C. R., Reimherr, F. W., Lisanby, S. H., Schwartz, T. L., Moreno, F. A., Dunner, D. L., Lesem, M. D., Thompson, P. M., Husain, M., Vine, C. J., Banov, M. D., Bernstein, L. P., Lehman, R. B., Brannon, G. E., Keepers, G. A., O'Reardon, J. P., Rudolph, R. L., & Bunker, M. (2013). Vagus nerve stimulation therapy randomized to different amounts of electrical charge for treatment-resistant depression: Acute and chronic effects. *Brain Stimulation*, *6*(4), 631–640. https://doi.org/10.1016/j.brs.2012.09.013

Aaronson, S. T., Sears, P., Ruvuna, F., Bunker, M., Conway, C. R., Dougherty, D. D., Reimherr, F. W., Schwartz, T. L., & Zajecka, J. M. (2017). A 5-Year observational study of patients with treatment-resistant depression treated with vagus nerve stimulation or treatment as usual: Comparison of response, remission, and suicidality. *The American Journal of Psychiatry*, *174*(7), 640–648. https://doi.org/10.1176/appi.ajp.2017.16010034

Abma, I. L., Rovers, M., & van der Wees, P. J. (2016). Appraising convergent validity of patient-reported outcome measures in systematic reviews: constructing hypotheses and interpreting outcomes. *BMC Research Notes*, *9*, 226. https://doi.org/10.1186/s13104-016-2034-2

Adams C. A. (2010). The probiotic paradox: live and dead cells are biological response modifiers. *Nutrition Research Reviews*, *23*(1), 37–46. https://doi.org/10.1017/S0954422410000090

Addolorato, G., Mirijello, A., D'Angelo, C., Leggio, L., Ferrulli, A., Abenavoli, L., Vonghia, L., Cardone, S., Leso, V., Cossari, A., Capristo, E., & Gasbarrini, G. (2008). State and trait anxiety and depression in patients affected by gastrointestinal diseases: Psychometric evaluation of 1641 patients referred to an internal medicine outpatient setting. *International Journal of Clinical Practice*, *62*(7), 1063–1069. https://doi.org/10.1111/j.1742-1241.2008.01763.x

Ahn, J., & Hayes, R. B. (2021). Environmental influences on the human microbiome and implications for noncommunicable disease. *Annual Review of Public Health*, *42*, 277–292. https://doi.org/10.1146/annurev-publhealth-012420-105020

Aiello, L. C. (1997). Brains and guts in human evolution: The expensive tissue hypothesis. *Brazilian Journal of Genetics*, *20*(1). https://doi.org/10.1590/S0100-84551997000100023

Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., Ota, M., Koga, N., Hattori, K., & Kunugi, H. (2016). Possible association of Bifidobacterium and Lactobacillus in the gut microbiota of patients with major depressive disorder. *Journal of Affective Disorders*, *202*, 254–257. https://doi.org/10.1016/j.jad.2016.05.038

Al-Awadhi, H., Dashti, N., Khanafer, M., Al-Mailem, D., Ali, N., & Radwan, S. (2013). Bias problems in culture-independent analysis of environmental bacterial communities: A representative study on hydrocarbonoclastic bacteria. *SpringerPlus*, *2*, 369. https://doi.org/10.1186/2193-1801-2-369

Al Bander, Z., Nitert, M. D., Mousa, A., & Naderpoor, N. (2020). The gut microbiota and inflammation: An overview. *International Journal of Environmental Research and Public Health*, *17*(20), 7618. https://doi.org/10.3390/ijerph17207618

Albillos, A., de Gottardi, A., & Rescigno, M. (2020). The gut-liver axis in liver disease: Pathophysiological basis for therapy. *Journal of Hepatology*, *72*(3), 558–577. https://doi.org/10.1016/j.jhep.2019.10.003

Allen, A. P., Dinan, T. G., Clarke, G., & Cryan, J. F. (2017). A psychology of the human brain-gut-microbiome axis. *Social and Personality Psychology Compass*, *11*(4), e12309. https://doi.org/10.1111/spc3.12309

Allen, B., & Nowak, M. A. (2013). Cooperation and the fate of microbial societies. *PLoS Biology*, *11*(4), e1001549. https://doi.org/10.1371/journal.pbio.1001549

Almeida, A., Mitchell, A. L., Boland, M., Forster, S. C., Gloor, G. B., Tarkowska, A., Lawley, T. D., & Finn, R. D. (2019). A new genomic blueprint of the human gut microbiota. *Nature*, *568*(7753), 499–504. https://doi.org/10.1038/s41586-019-0965-1

Almeida, C., Oliveira, R., Soares, R., & Barata, P. (2020). Influence of gut microbiota dysbiosis on brain function: A systematic review. *Porto Biomedical Journal*, *5*(2), 1-8. https://doi.org/10.1097/j.pbj.0000000000000059

Almond, M. (2013). Depression and inflammation: Examining the link. *Current Psychiatry*, *12*(6), 24-32.

Alisi, L., Cao, R., De Angelis, C., Cafolla, A., Caramia, F., Cartocci, G., Librando, A., & Fiorelli, M. (2019). The relationships between vitamin K and cognition: A review of current evidence. *Frontiers in Neurology*, *10*, 239. https://doi.org/10.3389/fneur.2019.00239

Amato, K. R., Yeoman, C. J., Cerda, G., Schmitt, C. A., Cramer, J. D., Miller, M. E., Gomez, A., Turner, T. R., Wilson, B. A., Stumpf, R. M., Nelson, K. E., White, B. A., Knight, R., & Leigh, S. R. (2015). Variable responses of human and non-human primate gut microbiomes to a Western diet. *Microbiome*, *3*, 53. https://doi.org/10.1186/s40168-015-0120-7

Anglin, R., Surette, M., Moayyedi, P., & Bercik, P. (2015). Lost in Translation: The Gut Microbiota in Psychiatric Illness. *Canadian journal of psychiatry. Revue Canadienne de Psychiatrie*, *60*(10), 460–463. https://doi.org/10.1177/070674371506001007

Angum, F., Khan, T., Kaler, J., Siddiqui, L., & Hussain, A. (2020). The prevalence of autoimmune disorders in women: A narrative review. *Cureus*, *12*(5), e8094. https://doi.org/10.7759/cureus.8094

Ansara E. D. (2020). Management of treatment-resistant generalized anxiety disorder. *The mental health clinician*, *10*(6), 326–334. https://doi.org/10.9740/mhc.2020.11.326

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., … Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346), 174–180. https://doi.org/10.1038/nature09944

Atkinson, R. C., & Shiffrin, R. M. (1968). Human memory: A proposed system and its control processes. In K. W. Spence & J. T. Spence, *The psychology of learning and motivation: II.* Academic Press. https://doi.org/10.1016/S0079-7421(08)60422-3

Avetisyan, M., Schill, E. M., & Heuckeroth, R. O. (2015). Building a second brain in the bowel. *The Journal of Clinical Investigation*, *125*(3), 899–907. https://doi.org/10.1172/JCI76307

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity*, *25*(3), 397–407. https://doi.org/10.1016/j.bbi.2010.10.023

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., Versalovic, J., Young, V., & Finlay, B. B. (2012). Defining a healthy human gut microbiome: current

concepts, future directions, and clinical applications. *Cell Host & Microbe*, *12*(5), 611–622. https://doi.org/10.1016/j.chom.2012.10.012

Bak, J., Shim, S. H., Kwon, Y. J., Lee, H. Y., Kim, J. S., Yoon, H., & Lee, Y. J. (2018). The association between suicide attempts and *Toxoplasma gondii* infection. *Clinical Psychopharmacology and Neuroscience*, *16*(1), 95–102. https://doi.org/10.9758/cpn.2018.16.1.95

Bandelow, B., & Michaelis, S. (2015). Epidemiology of anxiety disorders in the 21st century. *Dialogues in Clinical Neuroscience*, *17*(3), 327–335. https://doi.org/10.31887/DCNS.2015.17.3/bbandelow

Baquero, F., & Nombela, C. (2012). The microbiome as a human organ. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *18*(4), 2–4. https://doi.org/10.1111/j.1469-0691.2012.03916.x

Barratt, J. L., Harkness, J., Marriott, D., Ellis, J. T., & Stark, D. (2011). A review of Dientamoeba fragilis carriage in humans: several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. *Gut Microbes*, *2*(1), 3–12. https://doi.org/10.4161/gmic.2.1.14755

Bauer, M. A., Kainz, K., Carmona-Gutierrez, D., & Madeo, F. (2018). Microbial wars: Competition in ecological niches and within the microbiome. *Microbial Cell*, *5*(5), 215–219. https://doi.org/10.15698/mic2018.05.628

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Manual for the Beck depression inventory-II*. San Antonio, TX: Psychological Corporation.

Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, *157*(1), 121–141. https://doi.org/10.1016/j.cell.2014.03.011

Benedict, C., Vogel, H., Jonas, W., Woting, A., Blaut, M., Schürmann, A., & Cedernaes, J. (2016). Gut microbiota and glucometabolic alterations in response to recurrent partial sleep deprivation in normal-weight young individuals. *Molecular Metabolism*, *5*(12), 1175–1186. https://doi.org/10.1016/j.molmet.2016.10.003

Benton, D., Williams, C., & Brown, A. (2007). Impact of consuming a milk drink containing a probiotic on mood and cognition. *European Journal of Clinical Nutrition*, *61*(3), 355–361. https://doi.org/10.1038/sj.ejcn.1602546

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K. D., Verdu, E. F., & Collins, S. M. (2011). The intestinal microbiota affect central

levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, *141*(2), 599–609.e6093. https://doi.org/10.1053/j.gastro.2011.04.052

Berry, S. M., Broglio, K., Bunker, M., Jayewardene, A., Olin, B., & Rush, A. J. (2013). A patient-level meta-analysis of studies evaluating vagus nerve stimulation therapy for treatment-resistant depression. *Medical Devices*, *6*, 17–35. https://doi.org/10.2147/MDER.S41017

Bharti, R. & Grimm, D. G. (2021). Current challenges and best-practice protocols for microbiome analysis. *Briefings in Bioinformatics*, *22*(1), 178–193. https://doi.org/10.1093/bib/bbz155

Bianchi, V. E. (2018). The anti-Inflammatory effects of testosterone. *Journal of the Endocrine Society*, *3*(1), 91–107. https://doi.org/10.1210/js.2018-00186

Bischoff, S. C. (2011). 'Gut health': A new objective in medicine? *BMC Medicine, 9*, 1-14. https://doi.org/10.1186/1741-7015-9-24

Blaak, E. E., Canfora, E. E., Theis, S., Frost, G., Groen, A. K., Mithieux, G., Nauta, A., Scott, K., Stahl, B., van Harsselaar, J., van Tol, R., Vaughan, E. E., & Verbeke, K. (2020). Short chain fatty acids in human gut and metabolic health. *Beneficial Microbes*, *11*(5), 411–455. https://doi.org/10.3920/BM2020.0057

Blaser, M. J., Devkota, S., McCoy, K. D., Relman, D. A., Yassour, M., & Young, V. B. (2021). Lessons learned from the prenatal microbiome controversy. *Microbiome*, *9*(1), 8. https://doi.org/10.1186/s40168-020-00946-2

Blekhman, R., Goodrich, J. K., Huang, K., Sun, Q., Bukowski, R., Bell, J. T., Spector, T. D., Keinan, A., Ley, R. E., Gevers, D., & Clark, A. G. (2015). Host genetic variation impacts microbiome composition across human body sites. *Genome Biology*, *16*(1), 191. https://doi.org/10.1186/s13059-015-0759-1

Bliven, K. A., & Maurelli, A. T. (2016). Evolution of bacterial pathogens within the human host. *Microbiology Spectrum*, *4*(1), 10.1128/microbiolspec.VMBF-0017-2015. https://doi.org/10.1128/microbiolspec.VMBF-0017-2015

Blumstein, D. T., Levy, K., Mayer, E., & Harte, J. (2014). Gastrointestinal dysbiosis. *Evolution, Medicine, and Public Health*, *2014*(1), 163. https://doi.org/10.1093/emph/eou029

Bonaz, B., Bazin, T., & Pellissier, S. (2018). The vagus nerve at the interface of the microbiota-gut-brain axis. *Frontiers in Neuroscience*, *12*, 49. https://doi.org/10.3389/fnins.2018.00049

Bosi, A., Banfi, D., Bistoletti, M., Giaroni, C., & Baj, A. (2020). Tryptophan metabolites along the microbiota-gut-brain axis: An interkingdom communication system influencing the gut in health and disease. *International Journal of Tryptophan Research*, *13*, 1-25. https://doi.org/10.1177/1178646920928984

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., Bienenstock, J., & Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Broadbent, D. E. (1957). A mechanical model for human attention and immediate memory. *Psychological Review, 64*(3), 205–215. https://doi.org/10.1037/h0047313

Brown, K., DeCoffe, D., Molcan, E., & Gibson, D. L. (2012). Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients*, *4*(8), 1095–1119. https://doi.org/10.3390/nu4081095

Browne, H. P., Forster, S. C., Anonye, B. O., Kumar, N., Neville, B. A., Stares, M. D., Goulding, D., & Lawley, T. D. (2016). Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature*, *533*(7604), 543–546. https://doi.org/10.1038/nature17645

Browne, H. P., Neville, B. A., Forster, S. C., & Lawley, T. D. (2017). Transmission of the gut microbiota: Spreading of health. *Nature Reviews. Microbiology*, *15*(9), 531–543. https://doi.org/10.1038/nrmicro.2017.50

Browning, J. S., & Houseworth, J. H. (1953). Development of new symptoms following medical and surgical treatment for duodenal ulcer. *Psychosomatic Medicine*, *15*(4), 328–336. https://doi.org/10.1097/00006842-195307000-00006

Broussard, J. L., & Devkota, S. (2016). The changing microbial landscape of Western society: Diet, dwellings and discordance. *Molecular Metabolism*, *5*(9), 737–742. https://doi.org/10.1016/j.molmet.2016.07.007

Brumfield, K. D., Huq, A., Colwell, R. R., Olds, J. L., & Leddy, M. B. (2020). Microbial resolution of whole genome shotgun and 16S amplicon metagenomic sequencing using publicly available NEON data. *PloS One*, *15*(2), e0228899. https://doi.org/10.1371/journal.pone.0228899

Busi, S. B., de Nies, L., Habier, J., Wampach, L., Fritz, J. V., Heintz-Buschart, A., May, P., Halder, R., de Beauford, C., & Wilmes, P. (2021). Persistence of birth mode-dependent effects on gut

microbiome composition, immune system stimulation and antimicrobial resistance during the first year of life. *ISMA communications*, *1*(8), 1-12. https://doi.org/10.1038/s43705-021-00003-5

Butler, M. I., Mörkl, S., Sandhu, K. V., Cryan, J. F., & Dinan, T. G. (2019). The Gut Microbiome and Mental Health: What Should We Tell Our Patients?. *Canadian Journal of Psychiatry*, *64*(11), 747–760. https://doi.org/10.1177/0706743719874168

Bystritsky A. (2006). Treatment-resistant anxiety disorders. *Molecular Psychiatry*, *11*(9), 805–814. https://doi.org/10.1038/sj.mp.4001852

Campbell, W. H. & Rohrbaugh, R. M. (2006). *The biopsychosocial formulation manual: A guide for mental health professionals* (1st ed.). Routledge.

Cangelosi, G. A., & Meschke, J. S. (2014). Dead or alive: Molecular assessment of microbial viability. *Applied and Environmental Microbiology*, *80*(19), 5884–5891. https://doi.org/10.1128/AEM.01763-14

Capone, I., Marchetti, P., Ascierto, P. A., Malorni, W., & Gabriele, L. (2018). Sexual Dimorphism of Immune Responses: A New Perspective in Cancer Immunotherapy. *Frontiers in Immunology*, *9*, 552. https://doi.org/10.3389/fimmu.2018.00552

Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of Gastroenterology*, *28*(2), 203–209.

Carruthers, B. M., van de Sande, M. I., De Meirleir, K. L., Klimas, N. G., Broderick, G., Mitchell, T., Staines, D., Powles, A. C., Speight, N., Vallings, R., Bateman, L., Baumgarten-Austrheim, B., Bell, D. S., Carlo-Stella, N., Chia, J., Darragh, A., Jo, D., Lewis, D., Light, A. R., Marshall-Gradisnik, S., … Stevens, S. (2011). Myalgic encephalomyelitis: International Consensus Criteria. *Journal of Internal Medicine*, *270*(4), 327–338. https://doi.org/10.1111/j.1365-2796.2011.02428.x

Centers for Disease Control and Prevention. (2014). *Scientific nomenclature*. Retrieved November 16, 2020, from https://wwwnc.cdc.gov/eid/page/scientific-nomenclature

Chao, L., Liu, C., Sutthawongwadee, S., Li, Y., Lv, W., Chen, W., Yu, L., Zhou, J., Guo, A., Li, Z., & Guo, S. (2020). Effects of probiotics on depressive or anxiety variables in healthy participants under stress conditions or with a depressive or anxiety diagnosis: A meta-analysis of

randomized controlled trials. *Frontiers in Neurology*, *11*, 421. https://doi.org/10.3389/fneur.2020.00421

Chen, J. J., Zheng, P., Liu, Y. Y., Zhong, X. G., Wang, H. Y., Guo, Y. J., & Xie, P. (2018). Sex differences in gut microbiota in patients with major depressive disorder. *Neuropsychiatric Disease and Treatment*, *14*, 647–655. https://doi.org/10.2147/NDT.S159322

Cheng, M., Cao, L., & Ning, K. (2019). Microbiome big-data Mining and applications using single-cell technologies and metagenomics approaches toward precision medicine. *Frontiers in Genetics*, *10*, 972. https://doi.org/10.3389/fgene.2019.00972

Choi, J. M., Chung, G. E., Kang, S. J., Kwak, M. S., Yang, J. I., Park, B., & Yim, J. Y. (2021). Association Between Anxiety and Depression and Nonalcoholic Fatty Liver Disease. *Frontiers in Medicine*, *7*, 585618. https://doi.org/10.3389/fmed.2020.585618

Chow, J., Lee, S. M., Shen, Y., Khosravi, A., & Mazmanian, S. K. (2010). Host-bacterial symbiosis in health and disease. *Advances in Immunology*, *107*, 243–274. https://doi.org/10.1016/B978-0-12-381300-8.00008-3

Clapp, M., Aurora, N., Herrera, L., Bhatia, M., Wilen, E., & Wakefield, S. (2017). Gut microbiota's effect on mental health: The gut-brain axis. *Clinics and Practice*, *7*(4), 987. https://doi.org/10.4081/cp.2017.987

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, *18*(6), 666–673. https://doi.org/10.1038/mp.2012.77

Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. F., & Dinan, T. G. (2014). Minireview: Gut microbiota: the neglected endocrine organ. *Molecular Endocrinology*, *28*(8), 1221–1238. https://doi.org/10.1210/me.2014-1108

Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The impact of the gut microbiota on human health: an integrative view. *Cell*, *148*(6), 1258–1270. https://doi.org/10.1016/j.cell.2012.01.035

Clemente, L., Pasut, M., Carlet, R., Ruscio, M., & Fontana, F. (2021). Dientamoeba fragilis in the North-East of Italy: Prevalence study and treatment. *Parasitology International*, *80*, 102227. https://doi.org/10.1016/j.parint.2020.102227

Coertjens, L., Donche, V., De Maeyer, S., Vanthournout, G., & Van Petegem, P. (2017). To what degree does the missing-data technique influence the estimated growth in learning strategies over time? A tutorial example of sensitivity analysis for longitudinal data. *PloS One*, *12*(9), e0182615. https://doi.org/10.1371/journal.pone.0182615

Collado, M. C., Isolauri, E., Laitinen, K., & Salminen, S. (2008). Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *The American Journal of Clinical Nutrition*, *88*(4), 894–899. https://doi.org/10.1093/ajcn/88.4.894

Collins, J., Borojevic, R., Verdu, E. F., Huizinga, J. D., & Ratcliffe, E. M. (2014). Intestinal microbiota influence the early postnatal development of the enteric nervous system. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*, *26*(1), 98–107. https://doi.org/10.1111/nmo.12236

Collyer, R., Clancy, A., & Borody, T. (2020). Faecal microbiota transplantation alleviates symptoms of depression in individuals with irritable bowel syndrome: A case series. *Medicine in Microecology*, *6*(2020), 1-4. https://doi.org/10.1016/j.medmic.2020.100029

Conlon, M. A., & Bird, A. R. (2014). The impact of diet and lifestyle on gut microbiota and human health. *Nutrients*, *7*(1), 17–44. https://doi.org/10.3390/nu7010017

Coss-Adame, E., & Rao, S. S. (2014). Brain and gut interactions in irritable bowel syndrome: new paradigms and new understandings. *Current Gastroenterology Reports*, *16*(4), 379. https://doi.org/10.1007/s11894-014-0379-z

Costello, A. B., & Osborne, J. (2005). Best practices in exploratory factor analysis: Four recommendations for getting the most from your analysis. *Practical Assessment, Research, and Evaluation*, *10*, 1-10. https://doi.org/10.7275/jyj1-4868

Coulson, S., Butt, H., Vecchio, P., Gramotnev, H., & Vitetta, L. (2013). Green-lipped mussel extract (Perna canaliculus) and glucosamine sulphate in patients with knee osteoarthritis: therapeutic efficacy and effects on gastrointestinal microbiota profiles. *Inflammopharmacology*, *21*(1), 79–90. https://doi.org/10.1007/s10787-012-0146-4

Coyte, K. Z., & Rakoff-Nahoum, S. (2019). Understanding Competition and Cooperation within the Mammalian Gut Microbiome. *Current Biology*, *29*(11), 538–544. https://doi.org/10.1016/j.cub.2019.04.017

Cox, L. M., & Weiner, H. L. (2018). Microbiota signaling pathways that influence neurologic disease. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics*, *15*(1), 135–145. https://doi.org/10.1007/s13311-017-0598-8

Cryan, J. F. & Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the get microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*, 701-712. https://doi.org/10.1038/nrn3346

Cryan, J. F., & O'Mahony, S. M. (2011). The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*, *23*(3), 187–192. https://doi.org/10.1111/j.1365-2982.2010.01664.x

Cryan, J. F., O'Riordan, K. J., Cowan, C., Sandhu, K. V., Bastiaanssen, T., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'Connor, R., … Dinan, T. G. (2019). The Microbiota-Gut-Brain Axis. *Physiological Reviews*, *99*(4), 1877–2013. https://doi.org/10.1152/physrev.00018.2018

Cullen, C. M., Aneja, K. K., Beyhan, S., Cho, C. E., Woloszynek, S., Convertino, M., McCoy, S. J., Zhang, Y., Anderson, M. Z., Alvarez-Ponce, D., Smirnova, E., Karstens, L., Dorrestein, P. C., Li, H., Sen Gupta, A., Cheung, K., Powers, J. G., Zhao, Z., & Rosen, G. L. (2020). Emerging priorities for microbiome research. *Frontiers in Microbiology*, *11*, 136. https://doi.org/10.3389/fmicb.2020.00136

Cutter, G. R., & Liu, Y. (2012). Personalized medicine: The return of the house call?. *Neurology. Clinical Practice*, *2*(4), 343–351. https://doi.org/10.1212/CPJ.0b013e318278c328

Dama, M. S., Martinec Nováková, L., & Flegr, J. (2016). Do differences in Toxoplasma prevalence influence global variation in secondary sex ratio? Preliminary ecological regression study. *Parasitology*, *143*(9), 1193–1203. https://doi.org/10.1017/S0031182016000597

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: When the immune system subjugates the brain. *Nature Reviews. Neuroscience*, *9*(1), 46–56. https://doi.org/10.1038/nrn2297

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J., & Turnbaugh, P. J.

(2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563. https://doi.org/10.1038/nature12820

de Cena, J. A., Zhang, J., Deng, D., Damé-Teixeira, N., & Do, T. (2021). Low-abundant microorganisms: The human microbiome's dark matter, a scoping review. *Frontiers in Cellular and Infection Microbiology*, *11*, 689197. https://doi.org/10.3389/fcimb.2021.689197

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14691–14696. https://doi.org/10.1073/pnas.1005963107

De Hert, M., Detraux, J., & Vancampfort, D. (2018). The intriguing relationship between coronary heart disease and mental disorders. *Dialogues in Clinical Neuroscience*, *20*(1), 31–40. https://doi.org/10.31887/DCNS.2018.20.1/mdehert

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340. https://doi.org/10.1194/jlr.R036012

Deng, L., Yang, M., & Marcoulides, K. M. (2018). Structural equation modeling with many variables: A systematic review of issues and developments. *Frontiers in Psychology*, *9*, 580. https://doi.org/10.3389/fpsyg.2018.00580

Derogatis, L.R. (1992). *SCL-90-R: Administration, Scoring & Procedures Manual-II, for the R (Revised) Version and Other Instruments of the Psychopathology Rating Scale Series* (2nd ed.). Clinical Psychometric Research, Inc., Towson.

Derrien, M., Alvarez, A. S., & de Vos, W. M. (2019). The gut microbiota in the first decade of life. *Trends in Microbiology*, *27*(12), 997–1010. https://doi.org/10.1016/j.tim.2019.08.001

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry*, *19*(2), 146–148. https://doi.org/10.1038/mp.2013.65

Dethlefsen, L., McFall-Ngai, M. & Relman, D. (2007). An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature, 449,* 811–818. https://doi.org/10.1038/nature06245

Diamond, A. (2013). Executive functions. *Annual Review of Psychology*, *64*, 135–168. doi:10.1146/annurev-psych-113011-143750

Diggle, S. P., Griffin, A. S., Campbell, G. S., & West, S. A. (2007). Cooperation and conflict in quorum-sensing bacterial populations. *Nature*, *450*(7168), 411–414. https://doi.org/10.1038/nature06279

DiGiulio, D. B., Romero, R., Amogan, H. P., Kusanovic, J. P., Bik, E. M., Gotsch, F., Kim, C. J., Erez, O., Edwin, S., & Relman, D. A. (2008). Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: A molecular and culture-based investigation. *PloS One*, *3*(8), e3056. https://doi.org/10.1371/journal.pone.0003056

Dill-McFarland, K. A., Tang, Z. Z., Kemis, J. H., Kerby, R. L., Chen, G., Palloni, A., Sorenson, T., Rey, F. E., & Herd, P. (2019). Close social relationships correlate with human gut microbiota composition. *Scientific Reports*, *9*(1), 703. https://doi.org/10.1038/s41598-018-37298-9

Dinan, T. G., & Cryan, J. F. (2017). Brain-gut-microbiota axis and mental health. *Psychosomatic Medicine*, *79*(8), 920–926. https://doi.org/10.1097/PSY.0000000000000519

Donaldson, G. P., Lee, S. M., & Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nature Reviews. Microbiology*, *14*(1), 20–32. https://doi.org/10.1038/nrmicro3552

Donovan, P. D., Gonzalez, G., Higgins, D. G., Butler, G., & Ito, K. (2018). Identification of fungi in shotgun metagenomics datasets. *PloS One*, *13*(2), e0192898. https://doi.org/10.1371/journal.pone.0192898

Dukowicz, A. C., Lacy, B. E., & Levine, G. M. (2007). Small intestinal bacterial overgrowth: a comprehensive review. *Gastroenterology & Hepatology*, *3*(2), 112–122.

Emerson, J. B., Adams, R. I., Román, C., Brooks, B., Coil, D. A., Dahlhausen, K., Ganz, H. H., Hartmann, E. M., Hsu, T., Justice, N. B., Paulino-Lima, I. G., Luongo, J. C., Lymperopoulou, D. S., Gomez-Silvan, C., Rothschild-Mancinelli, B., Balk, M., Huttenhower, C., Nocker, A., Vaishampayan, P., & Rothschild, L. J. (2017). Schrödinger's microbes: Tools for distinguishing the living from the dead in microbial ecosystems. *Microbiome*, *5*(1), 86. https://doi.org/10.1186/s40168-017-0285-3

Enaud, R., Vandenborght, L. E., Coron, N., Bazin, T., Prevel, R., Schaeverbeke, T., Berger, P., Fayon, M., Lamireau, T., & Delhaes, L. (2018). The mycobiome: A neglected component in the

microbiota-gut-brain axis. *Microorganisms*, *6*(1), 22. https://doi.org/10.3390/microorganisms6010022

Engel G. L. (1977). The need for a new medical model: A challenge for biomedicine. *Science*, *196*(4286), 129–136. https://doi.org/10.1126/science.847460

Evans, J. M., Morris, L. S., & Marchesi, J. R. (2013). The gut microbiome: The role of a virtual organ in the endocrinology of the host. *The Journal of Endocrinology*, *218*(3), 37–47. https://doi.org/10.1530/JOE-13-0131

Farzi, A., Fröhlich, E. E., & Holzer, P. (2018). Gut microbiota and the neuroendocrine system. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics*, *15*(1), 5–22. https://doi.org/10.1007/s13311-017-0600-5

Felipo, V. (2013). Hepatic encephalopathy: effects of liver failure on brain function. *Nature reviews. Neuroscience*, *14*(12), 851–858. https://doi.org/10.1038/nrn3587

Feng, Y., Huang, Y., Wang, Y., Wang, P., Song, H., & Wang, F. (2019). Antibiotics induced intestinal tight junction barrier dysfunction is associated with microbiota dysbiosis, activated NLRP3 inflammasome and autophagy. *PloS One*, *14*(6), e0218384. https://doi.org/10.1371/journal.pone.0218384

Fenske, G. J., Ghimire, S., Antony, L., Christopher-Hennings, J., & Scaria, J. (2020). Integration of culture-dependent and independent methods provides a more coherent picture of the pig gut microbiome. *FEMS Microbiology Ecology*, *96*(3), fiaa022. https://doi.org/10.1093/femsec/fiaa022

Firth, J., Marx, W., Dash, S., Carney, R., Teasdale, S. B., Solmi, M., Stubbs, B., Schuch, F. B., Carvalho, A. F., Jacka, F., & Sarris, J. (2019). The effects of dietary improvement on symptoms of depression and anxiety: A meta-analysis of randomized controlled trials. *Psychosomatic Medicine*, *81*(3), 265–280. https://doi.org/10.1097/PSY.0000000000000673

Flegr, J. (2013). Influence of latent Toxoplasma infection on human personality, physiology and morphology: pros and cons of the Toxoplasma-human model in studying the manipulation hypothesis. *The Journal of Experimental Biology*, *216*(1), 127–133. https://doi.org/10.1242/jeb.073635

Flegr, J. & Kaňková, S. (2020). The effects of toxoplasmosis on sex ratio at birth. *Early Human Development*, *141*, 1-5. https://doi.org/10.1016/j.earlhumdev.2019.104874

Fond, G. B., Lagier, J. C., Honore, S., Lancon, C., Korchia, T., Sunhary De Verville, P. L., Llorca, P. M., Auquier, P., Guedj, E., & Boyer, L. (2020). Microbiota-orientated treatments for major depression and schizophrenia. *Nutrients*, *12*(4), 1024. https://doi.org/10.3390/nu12041024

Fortney, J. C., Pyne, J. M., Edlund, M. J., Stecker, T., Mittal, D., Robinson, D. E., & Henderson, K. L. (2011). Reasons for antidepressant nonadherence among veterans treated in primary care clinics. *The Journal of Clinical Psychiatry*, *72*(6), 827–834. https://doi.org/10.4088/JCP.09m05528blu

Foster, J. A., Rinaman, L., & Cryan, J. F. (2017). Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiology of Stress*, *7*, 124–136. https://doi.org/10.1016/j.ynstr.2017.03.001

Fragiadakis, G. K., Smits, S. A., Sonnenburg, E. D., Van Treuren, W., Reid, G., Knight, R., Manjurano, A., Changalucha, J., Dominguez-Bello, M. G., Leach, J., & Sonnenburg, J. L. (2019). Links between environment, diet, and the hunter-gatherer microbiome. *Gut Microbes*, *10*(2), 216–227. https://doi.org/10.1080/19490976.2018.1494103

Fransen, F., van Beek, A. A., Borghuis, T., Meijer, B., Hugenholtz, F., van der Gaast-de Jongh, C., Savelkoul, H. F., de Jonge, M. I., Faas, M. M., Boekschoten, M. V., Smidt, H., El Aidy, S., & de Vos, P. (2017). The impact of gut microbiota on gender-specific differences in immunity. *Frontiers in Immunology*, *8*, 754. https://doi.org/10.3389/fimmu.2017.00754

Fujimura, K. E., Slusher, N. A., Cabana, M. D., & Lynch, S. V. (2010). Role of the gut microbiota in defining human health. *Expert Review of Anti-Infective Therapy*, *8*(4), 435–454. https://doi.org/10.1586/eri.10.14

Fülling, C., Dinan, T. G., & Cryan, J. F. (2019). Gut microbe to brain signaling: What happens in vagus…. *Neuron*, *101*(6), 998–1002. https://doi.org/10.1016/j.neuron.2019.02.008

Furness, J. B., Callaghan, B. P., Rivera, L. R., & Cho, H. J. (2014). The enteric nervous system and gastrointestinal innervation: Integrated local and central control. *Advances in Experimental Medicine and Biology*, *817*, 39–71. https://doi.org/10.1007/978-1-4939-0897-4_3

Ganci, M., Butt, H., Tyrrell, J., Suleyman, E., & Ball, M. (2021). The effect of Blastocystis sp. and Dientamoeba fragilis on psychological symptom severity in a sample of clinically diverse males and females. *Current Psychology*. https://doi.org/10.1007/s12144-021-01700-z

Gao, A. & Martos, P. (2019). Log transformation and the effect on estimation, implication, and interpretation of mean and measurement uncertainty in microbial enumeration. *Journal of AOAC International, 102*(1), 233-238. https://doi.org/10.5740/jaoacint.18-0161

Gao, B. (2016). Basic liver immunology. *Cellular & Molecular Immunology*, *13*(3), 265–266. https://doi.org/10.1038/cmi.2016.09

Garcia, L. S. (2016). Dientamoeba fragilis; one of the neglected intestinal protozoa. *Journal of Clinical Microbiology*, *54*(9), 2243–2250. https://doi.org/10.1128/JCM.00400-16.

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., Macqueen, G., & Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, *60*(3), 307–317. https://doi.org/10.1136/gut.2009.202515

Gensollen, T., Iyer, S. S., Kasper, D. L., & Blumberg, R. S. (2016). How colonization by microbiota in early life shapes the immune system. *Science*, *352*(6285), 539–544. https://doi.org/10.1126/science.aad9378

Gershon M. D. (1999). The enteric nervous system: a second brain. *Hospital practice (1995)*, *34*(7). https://doi.org/10.3810/hp.1999.07.153

Gevers, D., Kugathasan, S., Denson, L. A., Vázquez-Baeza, Y., Van Treuren, W., Ren, B., Schwager, E., Knights, D., Song, S. J., Yassour, M., Morgan, X. C., Kostic, A. D., Luo, C., González, A., McDonald, D., Haberman, Y., Walters, T., Baker, S., Rosh, J., Stephens, M., … Xavier, R. J. (2014). The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host & Microbe*, *15*(3), 382–392. https://doi.org/10.1016/j.chom.2014.02.005

Ghoul, M., & Mitri, S. (2016). The ecology and evolution of microbial competition. *Trends in Microbiology*, *24*(10), 833–845. https://doi.org/10.1016/j.tim.2016.06.011

Gligorov, N., Azzouni, J., Lackey, D. P., & Zweig, A. (2013). Personal Identity: Our microbes, ourselves. In P. Rhodes, N. Gligorov, & A. P. Schwab (Eds.), *The human microbiome: ethical, legal and social concerns*. Oxford University Press.

Gluckman, P. D., Low, F. M., Buklijas, T., Hanson, M. A., & Beedle, A. S. (2011). How evolutionary principles improve the understanding of human health and disease. *Evolutionary Applications*, *4*(2), 249–263. https://doi.org/10.1111/j.1752-4571.2010.00164.x

Gouba, N., & Drancourt, M. (2015). Digestive tract mycobiota: A source of infection. *Medecine et Maladies Infectieuses*, *45*(1-2), 9–16. https://doi.org/10.1016/j.medmal.2015.01.007

Gravetter, F. & Wallnau, L. (2014) *Essentials of Statistics for the Behavioral Sciences* (8th ed.). Wadsworth.

Grenham, S., Clarke, G., Cryan, J. F., & Dinan, T. G. (2011). Brain-gut-microbe communication in health and disease. *Frontiers in Physiology*, *2*, 94. https://doi.org/10.3389/fphys.2011.00094

Greub, G. (2012). Culturomics: a new approach to study the human microbiome. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *18*(12), 1157–1159. https://doi.org/10.1111/1469-0691.12032

Griffin, A. S., West, S. A., & Buckling, A. (2004). Cooperation and competition in pathogenic bacteria. *Nature*, *430*(7003), 1024–1027. https://doi.org/10.1038/nature02744

Grigoryan, Z., Shen, M. J., Twardus, S. W., Beuttler, M. M., Chen, L. A., & Bateman-House, A. (2020). Fecal microbiota transplantation: Uses, questions, and ethics. *Medicine in Microecology*, *6*, 100027. https://doi.org/10.1016/j.medmic.2020.100027

Guo, P., Zhang, K., Ma, X., & He, P. (2020). *Clostridium* species as probiotics: Potentials and challenges. *Journal of Animal Science and Biotechnology*, *11*, 24. https://doi.org/10.1186/s40104-019-0402-1

Gupta, S., Allen-Vercoe, E., & Petrof, E. O. (2016). Fecal microbiota transplantation: In perspective. *Therapeutic Advances in Gastroenterology*, *9*(2), 229–239. https://doi.org/10.1177/1756283X15607414

Gurven, M., Kaplan, H., Winking, J., Eid Rodriguez, D., Vasunilashorn, S., Kim, J. K., Finch, C., & Crimmins, E. (2009). Inflammation and infection do not promote arterial aging and cardiovascular disease risk factors among lean horticulturalists. *PloS One*, *4*(8), e6590. https://doi.org/10.1371/journal.pone.0006590

Halford, J. C., & Blundell, J. E. (2000). Separate systems for serotonin and leptin in appetite control. *Annals of Medicine*, *32*(3), 222–232. https://doi.org/10.3109/07853890008998829

Hardt, J., Herke, M., & Leonhart, R. (2012). Auxiliary variables in multiple imputation in regression with missing X: A warning against including too many in small sample research. *BMC medical research methodology*, *12*, 184. https://doi.org/10.1186/1471-2288-12-184

Hasan, N., & Yang, H. (2019). Factors affecting the composition of the gut microbiota, and its modulation. *PeerJ*, *7*, e7502. https://doi.org/10.7717/peerj.7502

Hazen, C. & Shaver, P. (1987). Romantic love conceptualised as an attachment process. *Journal of Personality and Social Psychology*, *52*(3), 511-524.

Henderickx, J., Zwittink, R. D., van Lingen, R. A., Knol, J., & Belzer, C. (2019). The preterm gut microbiota: An inconspicuous challenge in nutritional neonatal care. *Frontiers in Cellular and Infection Microbiology*, *9*, 85. https://doi.org/10.3389/fcimb.2019.00085

Hibbing, M. E., Fuqua, C., Parsek, M. R., & Peterson, S. B. (2010). Bacterial competition: surviving and thriving in the microbial jungle. *Nature Reviews. Microbiology*, *8*(1), 15–25. https://doi.org/10.1038/nrmicro2259

Hiergeist, A., Gläsner, J., Reischl, U., & Gessner, A. (2015). Analyses of intestinal microbiota: Culture versus sequencing. *ILAR Journal*, *56*(2), 228–240. https://doi.org/10.1093/ilar/ilv017

Higgs, P., & Jones, I. R. (1999). Evolutionary psychology and health: Confronting an evolving paradigm. *Journal of Health Services Research & Policy*, *4*(3), 187–190. https://doi.org/10.1177/135581969900400312

Hillman, E. T., Lu, H., Yao, T., & Nakatsu, C. H. (2017). Microbial ecology along the gastrointestinal tract. *Microbes and Environments*, *32*(4), 300–313. https://doi.org/10.1264/jsme2.ME17017

Ho, S. C., Jacob, S. A., & Tangiisuran, B. (2017). Barriers and facilitators of adherence to antidepressants among outpatients with major depressive disorder: A qualitative study. *PloS One*, *12*(6), e0179290. https://doi.org/10.1371/journal.pone.0179290

Hoffmann, C., Dollive, S., Grunberg, S., Chen, J., Li, H., Wu, G. D., Lewis, J. D., & Bushman, F. D. (2013). Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PloS One*, *8*(6), e66019. https://doi.org/10.1371/journal.pone.0066019

Homann, C. M., Rossel, C., Dizzell, S., Bervoets, L., Simioni, J., Li, J., Gunn, E., Surette, M. G., de Souza, R. J., Mommers, M., Hutton, E. K., Morrison, K. M., Penders, J., van Best, N., & Stearns, J. C. (2021). Infants' first solid foods: Impact on gut microbiota development in two intercontinental cohorts. *Nutrients*, *13*(8), 2639. https://doi.org/10.3390/nu13082639

Hood, S. D., Hince, D. A., Robinson, H., Cirillo, M., Christmas, D., & Kaye, J. M. (2006). Serotonin regulation of the human stress response. *Psychoneuroendocrinology*, *31*(9), 1087–1097. https://doi.org/10.1016/j.psyneuen.2006.07.001

Hornef, M., & Penders, J. (2017). Does a prenatal bacterial microbiota exist?. *Mucosal Immunology*, *10*(3), 598–601. https://doi.org/10.1038/mi.2016.141

Hou, T. Y., Chiang-Ni, C., & Teng, S. H. (2019). Current status of MALDI-TOF mass spectrometry in clinical microbiology. *Journal of Food and Drug Analysis*, *27*(2), 404–414. https://doi.org/10.1016/j.jfda.2019.01.001

Howes, O. D., Thase, M. E., & Pillinger, T. (2021). Treatment resistance in psychiatry: State of the art and new directions. *Molecular Psychiatry,* 1-15. https://doi.org/10.1038/s41380-021-01200-3

Hoyle, R. H. (1995). The structural equation modeling approach: Basic concepts and fundamental issues. In R. H. Hoyle (Ed.), *Structural equation modeling: Concepts, issues, and applications* (pp. 1–15). Sage Publications, Inc.

Hu, J., Ly, J., Zhang, W., Huang, Y., Glover, V., Peter, I., Hurd, Y. L., & Nomura, Y. (2019). Microbiota of newborn meconium is associated with maternal anxiety experienced during

pregnancy. *Developmental Psychobiology*, *61*(5), 640–649. https://doi.org/10.1002/dev.21837

Huang, X., Liu, X., & Yu, Y. (2017). Depression and chronic liver diseases: Are there shared underlying mechanisms?. *Frontiers in Molecular Neuroscience*, *10*, 134. https://doi.org/10.3389/fnmol.2017.00134

Huang, C. H., Yu, X., & Liao, W. B. (2018). The expensive-tissue hypothesis in vertebrates: Gut microbiota effect, a review. *International Journal of Molecular Sciences*, *19*(6), 1792. https://doi.org/10.3390/ijms19061792

Hui, W., Li, T., Liu, W., Zhou, C., & Gao, F. (2019). Fecal microbiota transplantation for treatment of recurrent C. difficile infection: An updated randomized controlled trial meta-analysis. *PloS One*, *14*(1), e0210016. https://doi.org/10.1371/journal.pone.0210016

Hunt, R. H., Camilleri, M., Crowe, S. E., El-Omar, E. M., Fox, J. G., Kuipers, E. J., Malfertheiner, P., McColl, K. E., Pritchard, D. M., Rugge, M., Sonnenberg, A., Sugano, K., & Tack, J. (2015). The stomach in health and disease. *Gut*, *64*(10), 1650–1668. https://doi.org/10.1136/gutjnl-2014-307595

Huse, S. M., Young, V. B., Morrison, H. G., Antonopoulos, D. A., Kwon, J., Dalal, S., Arrieta, R., Hubert, N. A., Shen, L., Vineis, J. H., Koval, J. C., Sogin, M. L., Chang, E. B., & Raffals, L. E. (2014). Comparison of brush and biopsy sampling methods of the ileal pouch for assessment of mucosa-associated microbiota of human subjects. *Microbiome*, *2*(1), 5. https://doi.org/10.1186/2049-2618-2-5

Huseyin, C. E., Rubio, R. C., O'Sullivan, O., Cotter, P. D., & Scanlan, P. D. (2017). The fungal frontier: A comparative analysis of methods used in the study of the human gut mycobiome. *Frontiers in Microbiology*, *8*, 1432. https://doi.org/10.3389/fmicb.2017.01432

Hutter, T., Gimbert, C., Bouchard, F., & Lapointe, F. J. (2015). Being human is a gut feeling. *Microbiome*, *3*, 9. https://doi.org/10.1186/s40168-015-0076-7

Iacob, S., & Iacob, D. G. (2019). Infectious threats, the intestinal barrier, and its trojan horse: Dysbiosis. *Frontiers in Microbiology*, *10*, 1676. https://doi.org/10.3389/fmicb.2019.01676

Iliev, I. D., & Leonardi, I. (2017). Fungal dysbiosis: Immunity and interactions at mucosal barriers. *Nature Reviews. Immunology*, *17*(10), 635–646. https://doi.org/10.1038/nri.2017.55

Ingersoll, M. A. (2017). Sex differences shape the response to infectious diseases. *PLoS Pathogens*, *13*(12), e1006688. https://doi.org/10.1371/journal.ppat.1006688

Jacka, F. N., O'Neil, A., Opie, R., Itsiopoulos, C., Cotton, S., Mohebbi, M., Castle, D., Dash, S., Mihalopoulos, C., Chatterton, M. L., Brazionis, L., Dean, O. M., Hodge, A. M., & Berk, M.

(2017). A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES' trial). *BMC Medicine*, *15*(1), 23. https://doi.org/10.1186/s12916-017-0791-y

Jackson, M. L., Butt, H., Ball, M., Lewis, D. P., & Bruck, D. (2015). Sleep quality and the treatment of intestinal microbiota imbalance in Chronic Fatigue Syndrome: A pilot study. *Sleep Science*, *8*(3), 124–133. https://doi.org/10.1016/j.slsci.2015.10.001

Jaffe, D. H., Rive, B., & Denee, T. R. (2019). The humanistic and economic burden of treatment-resistant depression in Europe: A cross-sectional study. *BMC Psychiatry*, *19*, 247. https://doi.org/10.1186/s12888-019-2222-4

Jakobsdottir, G., Bjerregaard, J. H., Skovbjerg, H., & Nyman, M. (2013). Fasting serum concentration of short-chain fatty acids in subjects with microscopic colitis and celiac disease: no difference compared with controls, but between genders. *Scandinavian Journal of Gastroenterology*, *48*(6), 696–701. https://doi.org/10.3109/00365521.2013.786128

Jakobsen, J. C., Gluud, C., Wetterslev, J., & Winkel, P. (2017). When and how should multiple imputation be used for handling missing data in randomised clinical trials - a practical guide with flowcharts. *BMC Medical Research Methodology*, *17*(1), 162. https://doi.org/10.1186/s12874-017-0442-1

Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Nageshwar Reddy, D. (2015). Role of the normal gut microbiota. *World Journal of Gastroenterology*, *21*(29), 8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787

Jeong, H., Arif, B., Caetano-Anollés, G., Kim, K. M., & Nasir, A. (2019). Horizontal gene transfer in human-associated microorganisms inferred by phylogenetic reconstruction and reconciliation. *Scientific Reports*, *9*, 1-18. https://doi.org/10.1038/s41598-019-42227-5

Jia, F., & Wu, W. (2019). Evaluating methods for handling missing ordinal data in structural equation modeling. *Behavior Research Methods*, *51*(5), 2337–2355. https://doi.org/10.3758/s13428-018-1187-4

Jiang, H. Y., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., & Ruan, B. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, Behavior, and Immunity*, *48*, 186–194. https://doi.org/10.1016/j.bbi.2015.03.016

Jiang, H. Y., Zhang, X., Yu, Z. H., Zhang, Z., Deng, M., Zhao, J. H., & Ruan, B. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. *Journal of Psychiatric Research*, *104*, 130–136. https://doi.org/10.1016/j.jpsychires.2018.07.007

Jiang, X. & Hu, X. (2016). Microbiome Data Mining for Microbial Interactions and Relationships. In S. Pyne, B. Rao, & S. Rao (Eds.). *Big Data Analytics*. Springer. https://doi.org/10.1007/978-81-322-3628-3_12

Jiménez, E., Fernández, L., Marín, M. L., Martín, R., Odriozola, J. M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., & Rodríguez, J. M. (2005). Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Current Microbiology*, *51*(4), 270–274. https://doi.org/10.1007/s00284-005-0020-3

Johnson, K. V. A. (2020). Gut microbiome composition and diversity are related to human personality traits. *Human Microbiome Journal*, *15*, 1-15. https://doi.org/10.1016/j.humic.2019.100069

Kalin N. H. (2020). The critical relationship between anxiety and depression. *The American Journal of Psychiatry*, *177*(5), 365–367. https://doi.org/10.1176/appi.ajp.2020.20030305

Kang H. (2013). The prevention and handling of the missing data. *Korean Journal of Anesthesiology*, *64*(5), 402–406. https://doi.org/10.4097/kjae.2013.64.5.402

Kaňková, S., Kodym, P., Frynta, D., Vavrinová, R., Kubena, A., & Flegr, J. (2007). Influence of latent toxoplasmosis on the secondary sex ratio in mice. *Parasitology*, *134*(Pt 12), 1709–1717. https://doi.org/10.1017/S0031182007003253

Karahalios, A., Baglietto, L., Carlin, J. B., English, D. R., & Simpson, J. A. (2012). A review of the reporting and handling of missing data in cohort studies with repeated assessment of exposure measures. *BMC Medical Research Methodology*, *12*, 96. https://doi.org/10.1186/1471-2288-12-96

Kates, A. E., Jarrett, O., Skarlupka, J. H., Sethi, A., Duster, M., Watson, L., Suen, G., Poulsen, K., & Safdar, N. (2020). Household pet ownership and the microbial diversity of the human gut microbiota. *Frontiers in Cellular and Infection Microbiology*, *10*, 73. https://doi.org/10.3389/fcimb.2020.00073

Kaul, A., Davidov, O., & Peddada, S. D. (2017). Structural zeros in high-dimensional data with applications to microbiome studies. *Biostatistics (Oxford, England)*, *18*(3), 422–433. https://doi.org/10.1093/biostatistics/kxw053

Kazemi, A., Noorbala, A. A., Azam, K., Eskandari, M. H., & Djafarian, K. (2019). Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. *Clinical Nutrition*, *38*(2), 522–528. https://doi.org/10.1016/j.clnu.2018.04.010

Kelly, J. R., Clarke, G., Cryan, J. F., & Dinan, T. G. (2016). Brain-gut-microbiota axis: challenges for translation in psychiatry. *Annals of Epidemiology*, *26*(5), 366–372. https://doi.org/10.1016/j.annepidem.2016.02.008

Kelsen, J. R., & Wu, G. D. (2012). The gut microbiota, environment and diseases of modern society. *Gut Microbes*, *3*(4), 374–382. https://doi.org/10.4161/gmic.21333

Kennedy D. O. (2016). B vitamins and the brain: Mechanisms, dose and efficacy-A review. *Nutrients*, *8*(2), 68. https://doi.org/10.3390/nu8020068

Kim, H. Y. (2013). Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis. *Restorative Dentistry & Endodontics*, *38*(1), 52–54. https://doi.org/10.5395/rde.2013.38.1.52

Kim, D. Y., & Camilleri, M. (2000). Serotonin: A mediator of the brain-gut connection. *The American Journal of Gastroenterology*, *95*(10), 2698–2709. https://doi.org/10.1111/j.1572-0241.2000.03177.x

Kim, H. N., Yun, Y., Ryu, S., Chang, Y., Kwon, M. J., Cho, J., Shin, H., & Kim, H. L. (2018). Correlation between gut microbiota and personality in adults: A cross-sectional study. *Brain, Behavior, and Immunity*, *69*, 374–385. https://doi.org/10.1016/j.bbi.2017.12.012

King, C. H., Desai, H., Sylvetsky, A. C., LoTempio, J., Ayanyan, S., Carrie, J., Crandall, K. A., Fochtman, B. C., Gasparyan, L., Gulzar, N., Howell, P., Issa, N., Krampis, K., Mishra, L., Morizono, H., Pisegna, J. R., Rao, S., Ren, Y., Simonyan, V., Smith, K., … Mazumder, R. (2019). Baseline human gut microbiota profile in healthy people and standard reporting template. *PloS one*, *14*(9), e0206484. https://doi.org/10.1371/journal.pone.0206484

Kinser, P. A., & Robins, J. L. (2013). Control group design: Enhancing rigor in research of mind-body therapies for depression. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 140467. https://doi.org/10.1155/2013/140467

Klimova, B., Dziuba, S., & Cierniak-Emerych, A. (2020). The effect of healthy diet on cognitive performance among healthy seniors - a mini review. *Frontiers in Human Neuroscience*, *14*, 325. https://doi.org/10.3389/fnhum.2020.00325

Konturek, P. C., Harsch, I. A., Konturek, K., Schink, M., Konturek, T., Neurath, M. F., & Zopf, Y. (2018). Gut-Liver Axis: How Do Gut Bacteria Influence the Liver?. *Medical Sciences (Basel, Switzerland)*, *6*(3), 79. https://doi.org/10.3390/medsci6030079

Kopp, W. (2019). How western diet and lifestyle drive the pandemic of obesity and civilization diseases. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *12*, 2221–2236. https://doi.org/10.2147/DMSO.S216791

Koren, O., Goodrich, J. K., Cullender, T. C., Spor, A., Laitinen, K., Bäckhed, H. K., Gonzalez, A., Werner, J. J., Angenent, L. T., Knight, R., Bäckhed, F., Isolauri, E., Salminen, S., & Ley, R. E. (2012). Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell*, *150*(3), 470–480. https://doi.org/10.1016/j.cell.2012.07.008

Kumar, P., & Magon, N. (2012). Hormones in pregnancy. *Nigerian Medical Journal: Journal of the Nigeria Medical Association*, *53*(4), 179–183. https://doi.org/10.4103/0300-1652.107549

Lagier, J. C., Armougom, F., Million, M., Hugon, P., Pagnier, I., Robert, C., Bittar, F., Fournous, G., Gimenez, G., Maraninchi, M., Trape, J. F., Koonin, E. V., La Scola, B., & Raoult, D. (2012). Microbial culturomics: Paradigm shift in the human gut microbiome study. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *18*(12), 1185–1193. https://doi.org/10.1111/1469-0691.12023

Lagier, J. C., Dubourg, G., Million, M., Cadoret, F., Bilen, M., Fenollar, F., Levasseur, A., Rolain, J. M., Fournier, P. E., & Raoult, D. (2018). Culturing the human microbiota and culturomics. *Nature Reviews. Microbiology*, *16*, 540–550. https://doi.org/10.1038/s41579-018-0041-0

Lagier, J. C., Hugon, P., Khelaifia, S., Fournier, P. E., La Scola, B., & Raoult, D. (2015). The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. *Clinical Microbiology Reviews*, *28*(1), 237–264. https://doi.org/10.1128/CMR.00014-14

Lai, W. T., Deng, W. F., Xu, S. X., Zhao, J., Xu, D., Liu, Y. H., Guo, Y. Y., Wang, M. B., He, F. S., Ye, S. W., Yang, Q. F., Liu, T. B., Zhang, Y. L., Wang, S., Li, M. Z., Yang, Y. J., Xie, X. H., & Rong, H. (2021). Shotgun metagenomics reveals both taxonomic and tryptophan pathway differences of gut microbiota in major depressive disorder patients. *Psychological Medicine*, *51*(1), 90–101. https://doi.org/10.1017/S0033291719003027

Lappinga, P. J., Abraham, S. C., Murray, J. A., Vetter, E. A., Patel, R., & Wu, T. T. (2010). Small intestinal bacterial overgrowth: histopathologic features and clinical correlates in an underrecognized entity. *Archives of pathology & laboratory medicine*, *134*(2), 264–270. https://doi.org/10.5858/134.2.264

Lara-Hidalgo, C. E., Hernández-Sánchez, H., Hernández-Rodríguez, C., & Dorantes-Álvarez, L. (2017). Yeasts in fermented foods and their probiotic potential. *Austin Journal of Nutrition and Metabolism, 4*(1), 1045.

Lau, J. T., Whelan, F. J., Herath, I., Lee, C. H., Collins, S. M., Bercik, P., & Surette, M. G. (2016). Capturing the diversity of the human gut microbiota through culture-enriched molecular profiling. *Genome Medicine*, *8*, 72. https://doi.org/10.1186/s13073-016-0327-7

Laursen, M. F., Zachariassen, G., Bahl, M. I., Bergström, A., Høst, A., Michaelsen, K. F., & Licht, T. R. (2015). Having older siblings is associated with gut microbiota development during early childhood. *BMC Microbiology*, *15*, 154. https://doi.org/10.1186/s12866-015-0477-6

Lee, K. J., Tilling, K. M., Cornish, R. P., Little, R., Bell, M. L., Goetghebeur, E., Hogan, J. W., Carpenter, J. R., & STRATOS initiative (2021). Framework for the treatment and reporting of missing data in observational studies: The treatment and reporting of missing data in observational studies framework. *Journal of Clinical Epidemiology*, *134*, 79–88. https://doi.org/10.1016/j.jclinepi.2021.01.008

Leeming, E. R., Johnson, A. J., Spector, T. D., & Le Roy, C. I. (2019). Effect of diet on the gut microbiota: Rethinking intervention duration. *Nutrients*, *11*(12), 2862. https://doi.org/10.3390/nu11122862

Leite, G., Weitsman, S., Parodi, G., Celly, S., Sedighi, R., Sanchez, M., Morales, W., Villanueva-Millan, M. J., Barlow, G. M., Mathur, R., Lo, S. K., Jamil, L. H., Paski, S., Rezaie, A., & Pimentel, M. (2020). Mapping the segmental microbiomes in the human small bowel in comparison with stool: A REIMAGINE study. *Digestive Diseases and Sciences*, *65*(9), 2595–2604. https://doi.org/10.1007/s10620-020-06173-x

Lepczyńska, M., Białkowska, J., Dzika, E., Piskorz-Ogórek, K., & Korycińska, J. (2017). Blastocystis: How do specific diets and human gut microbiota affect its development and pathogenicity?. *European Journal of Clinical Microbiology & Infectious Diseases*, *36*(9), 1531–1540. https://doi.org/10.1007/s10096-017-2965-0

Lew, L. C., Hor, Y. Y., Yusoff, N., Choi, S. B., Yusoff, M., Roslan, N. S., Ahmad, A., Mohammad, J., Abdullah, M., Zakaria, N., Wahid, N., Sun, Z., Kwok, L. Y., Zhang, H., & Liong, M. T. (2019). Probiotic Lactobacillus plantarum P8 alleviated stress and anxiety while enhancing memory and cognition in stressed adults: A randomised, double-blind, placebo-controlled study. *Clinical Nutrition*, *38*(5), 2053–2064. https://doi.org/10.1016/j.clnu.2018.09.010

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., & Gordon, J. I. (2008). Worlds within worlds: Evolution of the vertebrate gut microbiota. *Nature Reviews. Microbiology*, *6*(10), 776–788. https://doi.org/10.1038/nrmicro1978

Li, H., Zhou, M., Zhao, A., & Jia, W. (2009). Traditional Chinese medicine: balancing the gut ecosystem. *Phytotherapy Research: PTR*, *23*(9), 1332–1335. https://doi.org/10.1002/ptr.2590

Lim, G. Y., Tam, W. W., Lu, Y., Ho, C. S., Zhang, M. W., & Ho, R. C. (2018). Prevalence of depression in the community from 30 countries between 1994 and 2014. *Scientific Reports*, *8*(1), 2861. https://doi.org/10.1038/s41598-018-21243-x

Liu, Y. Z., Wang, Y. X., & Jiang, C. L. (2017). Inflammation: The common pathway of stress-related diseases. *Frontiers in Human Neuroscience*, *11*, 316. https://doi.org/10.3389/fnhum.2017.00316

Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine*, *8*(1), 51. https://doi.org/10.1186/s13073-016-0307-y

Lobato, L., Bethony, J. M., Pereira, F. B., Grahek, S. L., Diemert, D., & Gazzinelli, M. F. (2014). Impact of gender on the decision to participate in a clinical trial: A cross-sectional study. *BMC Public Health*, *14*, 1156. https://doi.org/10.1186/1471-2458-14-1156

Loke, P., & Lim, Y. A. (2015). Helminths and the microbiota: parts of the hygiene hypothesis. *Parasite Immunology*, *37*(6), 314–323. https://doi.org/10.1111/pim.12193

Lokmer, A., Cian, A., Froment, A., Gantois, N., Viscogliosi, E., Chabé, M., & Ségurel, L. (2019). Use of shotgun metagenomics for the identification of protozoa in the gut microbiota of healthy individuals from worldwide populations with various industrialization levels. *PloS One*, *14*(2), e0211139. https://doi.org/10.1371/journal.pone.0211139

Lopetuso, L. R., Scaldaferri, F., Petito, V., & Gasbarrini, A. (2013). Commensal Clostridia: Leading players in the maintenance of gut homeostasis. *Gut Pathogens*, *5*(1), 23. https://doi.org/10.1186/1757-4749-5-23

Loughrey, D. G., Lavecchia, S., Brennan, S., Lawlor, B. A., & Kelly, M. E. (2017). The impact of the Mediterranean diet on the cognitive functioning of healthy older adults: A systematic review and meta-analysis. *Advances in Nutrition*, *8*(4), 571–586. https://doi.org/10.3945/an.117.015495

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, *489*(7415), 220–230. https://doi.org/10.1038/nature11550

Lukeš, J., Stensvold, C. R., Jirků-Pomajbíková, K., & Wegener Parfrey, L. (2015). Are human intestinal eukaryotes beneficial or commensals?. *PLoS Pathogens*, *11*(8), e1005039. https://doi.org/10.1371/journal.ppat.1005039

Lyte, M. (2010). The microbial organ in the gut as a driver of homeostasis and disease. *Medical Hypotheses*, *74*(4), 634–638. https://doi.org/10.1016/j.mehy.2009.10.025

Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, *33*(8), 574–581. https://doi.org/10.1002/bies.201100024

Ma, J., Li, Z., Zhang, W., Zhang, C., Zhang, Y., Mei, H., Zhuo, N., Wang, H., Wang, L., & Wu, D. (2020). Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: a study of 91 term infants. *Scientific Reports*, *10*(1), 15792. https://doi.org/10.1038/s41598-020-72635-x

Madley-Dowd, P., Hughes, R., Tilling, K., & Heron, J. (2019). The proportion of missing data should not be used to guide decisions on multiple imputation. *Journal of clinical epidemiology*, *110*, 63–73. https://doi.org/10.1016/j.jclinepi.2019.02.016

Maehata, H., Arai, S., Iwabuchi, N., & Abe, F. (2021). Immuno-modulation by heat-killed *Lacticaseibacillus paracasei* MCC1849 and its application to food products. *International Journal of Immunopathology and Pharmacology*, *35*, 20587384211008291. https://doi.org/10.1177/20587384211008291

Madison, A., & Kiecolt-Glaser, J. K. (2019). Stress, depression, diet, and the gut microbiota: human-bacteria interactions at the core of psychoneuroimmunology and nutrition. *Current Opinion in Behavioral Sciences*, *28*, 105–110. https://doi.org/10.1016/j.cobeha.2019.01.011

Mangiola, S., Thomas, E. A., Modrák, M., Vehtari, A., & Papenfuss, A. T. (2021). Probabilistic outlier identification for RNA sequencing generalized linear models. *NAR Genomics and Bioinformatics*, *3*(1), lqab005. https://doi.org/10.1093/nargab/lqab005

Mapou, R. L. (1995). A cognitive framework for neuropsychological assessment. In R. L. Mapou & J. Spector (Eds.*), Clinical neuropsychological Assessment: A cognitive approach* (pp. 295-337). Plenum Press.

Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D., Hirschfield, G. M., Hold, G., Quraishi, M. N., Kinross, J., Smidt, H., Tuohy, K. M., Thomas, L. V., Zoetendal, E. G., & Hart, A. (2016). The gut microbiota and host health: a new clinical frontier. *Gut*, *65*(2), 330–339. https://doi.org/10.1136/gutjnl-2015-309990

Marchesi, J. R. & Ravel, J. (2015). The vocabulary of microbiome research: A proposal. *Microbiome*, *3*(31), 1-3. https://doi.org/10.1186/s40168-015-0094-5

Markle, J. G., Frank, D. N., Mortin-Toth, S., Robertson, C. E., Feazel, L. M., Rolle-Kampczyk, U., von Bergen, M., McCoy, K. D., Macpherson, A. J., & Danska, J. S. (2013). Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science*, *339*(6123), 1084–1088. https://doi.org/10.1126/science.1233521

Markovitz, A. A., Simanek, A. M., Yolken, R. H., Galea, S., Koenen, K. C., Chen, S., & Aiello, A. E. (2015). Toxoplasma gondii and anxiety disorders in a community-based sample. *Brain, Behavior, and Immunity*, *43*, 192–197. https://doi.org/10.1016/j.bbi.2014.08.001

Marks, D. M., Shah, M. J., Patkar, A. A., Masand, P. S., Park, G. Y., & Pae, C. U. (2009). Serotonin-norepinephrine reuptake inhibitors for pain control: Premise and promise. *Current Neuropharmacology*, *7*(4), 331–336. https://doi.org/10.2174/157015909790031201

Marques, T. M., Cryan, J. F., Shanahan, F., Fitzgerald, G. F., Ross, R. P., Dinan, T. G., & Stanton, C. (2014). Gut microbiota modulation and implications for host health: Dietary stratergies to influence the gut-brain axis. *Innovative Food Science & Emerging Technologies*, *22*, 239-247. https://doi.org/10.1016/j.ifset.2013.10.016

Martin, R., Makino, H., Cetinyurek Yavuz, A., Ben-Amor, K., Roelofs, M., Ishikawa, E., Kubota, H., Swinkels, S., Sakai, T., Oishi, K., Kushiro, A., & Knol, J. (2016). Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. *PloS one*, *11*(6), e0158498. https://doi.org/10.1371/journal.pone.0158498

Matijašić, M., Meštrović, T., Paljetak, H. Č., Perić, M., Barešić, A., & Verbanac, D. (2020). Gut microbiota beyond bacteria – mycobiome, virome, archaeome, and eukaryotic parasites in IBD. *International Journal of Molecular Sciences*, *21*(8), 2668. https://doi.org/10.3390/ijms21082668

Mayer, E. A. (2011). Gut feelings: the emerging biology of gut-brain communication. *Nature Reviews. Neuroscience*, *12*(8), 453–466. https://doi.org/10.1038/nrn3071

McAllister-Williams, R. H., Sousa, S., Kumar, A., Greco, T., Bunker, M. T., Aaronson, S. T., Conway, C. R., & Rush, A. J. (2020). The effects of vagus nerve stimulation on the course and outcomes of patients with bipolar disorder in a treatment-resistant depressive episode: A 5-year prospective registry. *International Journal of Bipolar Disorders*, *8*(1), 13. https://doi.org/10.1186/s40345-020-0178-4

McCrae, R. R., Kurtz, J. E., Yamagata, S., & Terracciano, A. (2011). Internal consistency, retest reliability, and their implications for personality scale validity. *Personality and social psychology review: An Official Journal of the Society for Personality and Social Psychology, Inc*, *15*(1), 28–50. https://doi.org/10.1177/1088868310366253

McDonald, D., Ackermann, G., Khailova, L., Baird, C., Heyland, D., Kozar, R., Lemieux, M., Derenski, K., King, J., Vis-Kampen, C., Knight, R., & Wischmeyer, P. E. (2016). Extreme dysbiosis of the microbiome in critical illness. *mSphere*, *1*(4), e00199-16. https://doi.org/10.1128/mSphere.00199-16

Meneses, A., & Liy-Salmeron, G. (2012). Serotonin and emotion, learning and memory. *Reviews in the Neurosciences*, *23*(5-6), 543–553. https://doi.org/10.1515/revneuro-2012-0060

Messaoudi, M., Violle, N., Bisson, J. F., Desor, D., Javelot, H., & Rougeot, C. (2011). Beneficial psychological effects of a probiotic formulation (Lactobacillus helveticus R0052 and

Bifidobacterium longum R0175) in healthy human volunteers. *Gut Microbes*, *2*(4), 256–261. https://doi.org/10.4161/gmic.2.4.16108

Metcalf, L. & Casey, W. (2016). *Cybersecurity and applied mathematics*. Synergess.

Meyyappan, A. C., Forth, E., Wallace, C., & Milev, R. (2020). Effect of fecal microbiota transplant on symptoms of psychiatric disorders: a systematic review. *BMC Psychiatry*, *20*(1), 299. https://doi.org/10.1186/s12888-020-02654-5

Miguel, L., Salvador, F., Sulleiro, E., Sánchez-Montalvá, A., Molina-Morant, D., López, I., & Molina, I. (2018). Clinical and epidemiological characteristics of patients with *Dientamoeba fragilis* Infection. *The American Journal of Tropical Medicine and Hygiene*, *99*(5), 1170–1173. https://doi.org/10.4269/ajtmh.18-0433

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., Belzer, C., Delgado Palacio, S., Arboleya Montes, S., Mancabelli, L., Lugli, G. A., Rodriguez, J. M., Bode, L., de Vos, W., Gueimonde, M., Margolles, A., van Sinderen, D., & Ventura, M. (2017). The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews*, *81*(4), e00036-17. https://doi.org/10.1128/MMBR.00036-17

Miller A. H. (2020). Beyond depression: The expanding role of inflammation in psychiatric disorders. *World Psychiatry: Official Journal of the World Psychiatric Association*, *19*(1), 108–109. https://doi.org/10.1002/wps.20723

Miller, G. A. (2003). The cognitive revolution: A historical perspective. *Trends in Cognitive Sciences, 7*(3), 141–144. https://doi.org/10.1016/S1364-6613(03)00029-9

Minihane, A. M., Vinoy, S., Russell, W. R., Baka, A., Roche, H. M., Tuohy, K. M., Teeling, J. L., Blaak, E. E., Fenech, M., Vauzour, D., McArdle, H. J., Kremer, B. H., Sterkman, L., Vafeiadou, K., Benedetti, M. M., Williams, C. M., & Calder, P. C. (2015). Low-grade inflammation, diet composition and health: current research evidence and its translation. *The British Journal of Nutrition*, *114*(7), 999–1012. https://doi.org/10.1017/S0007114515002093

Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. *Frontiers in Cellular Neuroscience*, *13*, 363. https://doi.org/10.3389/fncel.2019.00363

Miskinyte, M., Sousa, A., Ramiro, R. S., de Sousa, J. A., Kotlinowski, J., Caramalho, I., Magalhães, S., Soares, M. P., & Gordo, I. (2013). The genetic basis of Escherichia coli pathoadaptation to

macrophages. *PLoS Pathogens*, *9*(12), e1003802. https://doi.org/10.1371/journal.ppat.1003802

Moeller, A. H., Li, Y., Mpoudi Ngole, E., Ahuka-Mundeke, S., Lonsdorf, E. V., Pusey, A. E., Peeters, M., Hahn, B. H., & Ochman, H. (2014). Rapid changes in the gut microbiome during human evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(46), 16431–16435. https://doi.org/10.1073/pnas.1419136111

Moieni, M., & Eisenberger, N. I. (2018). Effects of inflammation on social processes and implications for health. *Annals of the New York Academy of Sciences*, *1428*(1), 5–13. https://doi.org/10.1111/nyas.13864

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The microbiome: Stress, health and disease. *Mammalian Genome: Official Journal of the International Mammalian Genome Society*, *25*(1-2), 49–74. https://doi.org/10.1007/s00335-013-9488-5

Mosca, A., Leclerc, M., & Hugot, J. P. (2016). Gut microbiota diversity and human diseases: Should we reintroduce key predators in our ecosystem?. *Frontiers in Microbiology*, *7*, 455. https://doi.org/10.3389/fmicb.2016.00455

Mu, C., Yang, Y., & Zhu, W. (2016). Gut Microbiota: The Brain Peacekeeper. *Frontiers in Microbiology*, *7*, 345. https://doi.org/10.3389/fmicb.2016.00345

Mu, Q., Kirby, J., Reilly, C. M., & Luo, X. M. (2017). Leaky gut as a danger signal for autoimmune diseases. *Frontiers in Immunology*, *8*, 598. https://doi.org/10.3389/fimmu.2017.00598

Mueller, N. T., Bakacs, E., Combellick, J., Grigoryan, Z., & Dominguez-Bello, M. G. (2015). The infant microbiome development: mom matters. *Trends in Molecular Medicine*, *21*(2), 109–117. https://doi.org/10.1016/j.molmed.2014.12.002

Mulak, A., Taché, Y., & Larauche, M. (2014). Sex hormones in the modulation of irritable bowel syndrome. *World Journal of Gastroenterology*, *20*(10), 2433–2448. https://doi.org/10.3748/wjg.v20.i10.2433

Müller, C. P., & Homberg, J. R. (2015). The role of serotonin in drug use and addiction. *Behavioural Brain Research*, *277*, 146–192. https://doi.org/10.1016/j.bbr.2014.04.007

Nagpal, R., & Yadav, H. (2017). Bacterial translocation from the gut to the distant organs: An overview. *Annals of Nutrition & Metabolism*, *71 Suppl 1*, 11–16. https://doi.org/10.1159/000479918

Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linløkken, A., Wilson, R., & Rudi, K. (2014). Correlation between the human fecal microbiota and depression. *Neurogastroenterology*

and Motility: The Official Journal of the European Gastrointestinal Motility Society, *26*(8), 1155–1162. https://doi.org/10.1111/nmo.12378

Newell, A. (1973). You can't play 20 questions with nature and win: Projective comments on the papers of this symposium. In W. G. Chase (Ed.), *Visual information processing* (pp. 283–308). New York: Academic Press.

Newell, A. (1990). *Unified theories of cognition.* Harvard University Press.

Newman, D. A., & Cottrell, J. M. (2015). Missing data bias: Exactly how bad is pairwise deletion? In C. E. Lance & R. J. Vandenberg (Eds.), *More statistical and methodological myths and urban legends* (pp. 133–161). Routledge/Taylor & Francis Group.

Nguyen, T. T., Kosciolek, T., Maldonado, Y., Daly, R. E., Martin, A. S., McDonald, D., Knight, R., & Jeste, D. V. (2019). Differences in gut microbiome composition between persons with chronic schizophrenia and healthy comparison subjects. *Schizophrenia Research*, *204*, 23–29. https://doi.org/10.1016/j.schres.2018.09.014

Nichols, R. G., Cai, J., Murray, I. A., Koo, I., Smith, P. B., Perdew, G. H., & Patterson, A. D. (2018). Structural and functional analysis of the gut microbiome for toxicologists. *Current Protocols in Toxicology*, *78*(1), e54. https://doi.org/10.1002/cptx.54

Nourrisson, C., Scanzi, J., Pereira, B., NkoudMongo, C., Wawrzyniak, I., Cian, A., Viscogliosi, E., Livrelli, V., Delbac, F., Dapoigny, M., & Poirier, P. (2014). Blastocystis is associated with decrease of fecal microbiota protective bacteria: comparative analysis between patients with irritable bowel syndrome and control subjects. *PloS One*, *9*(11), e111868. https://doi.org/10.1371/journal.pone.0111868

Novakovic, M., Rout, A., Kingsley, T., Kirchoff, R., Singh, A., Verma, V., Kant, R., & Chaudhary, R. (2020). Role of gut microbiota in cardiovascular diseases. *World Journal of Cardiology*, *12*(4), 110–122. https://doi.org/10.4330/wjc.v12.i4.110

O'Callaghan, T. F., Ross, R. P., Stanton, C., & Clarke, G. (2016). The gut microbiome as a virtual endocrine organ with implications for farm and domestic animal endocrinology. *Domestic Animal Endocrinology*, *56*, 44–55. https://doi.org/10.1016/j.domaniend.2016.05.003

O'Dea K. (1991). Cardiovascular disease risk factors in Australian aborigines. *Clinical and Experimental Pharmacology & Physiology*, *18*(2), 85–88. https://doi.org/10.1111/j.1440-1681.1991.tb01412.x

Odamaki, T., Kato, K., Sugahara, H., Hashikura, N, Takahashi, S., Xiao, J. Z., Abe, F., & Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: A cross-sectional study. *BMC Microbiology*, *16*, 1-12. https://doi.org/10.1186/s12866-016-0708-5

Ohata, A., Usami, M., & Miyoshi, M. (2005). Short-chain fatty acids alter tight junction permeability in intestinal monolayer cells via lipoxygenase activation. *Nutrition*, *21*(7-8), 838–847. https://doi.org/10.1016/j.nut.2004.12.004

Okada, H., Kuhn, C., Feillet, H., & Bach, J. F. (2010). The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clinical and Experimental Immunology*, *160*(1), 1–9. https://doi.org/10.1111/j.1365-2249.2010.04139.x

Oliveira-Arbex, A. P., David, É. B., Cacciò, S. M., Fonseca, C., Martin, J. G., Kurokawa, C. S., Tosini, F., Souza Neto, J. A., & Guimarães, S. (2021). Prevalence and genetic characterization of Dientamoeba fragilis in asymptomatic children attending daycare centers. *Revista do Instituto de Medicina Tropical de Sao Paulo*, *63*, e39. https://doi.org/10.1590/S1678-9946202163039

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research*, *277*, 32–48. https://doi.org/10.1016/j.bbr.2014.07.027

Omenn, G. S. (2010). Evolution and public health. *Proceeding of the National Academy of Sciences*, *107* (Suppl 1), 1702-1709. https://doi.org/10.1073/pnas.0906198106

Organisation for Economic Cooperation and Development. (2021). *Mental Health*. Retrieved July 9 from https://www.oecd.org/els/health-systems/mental-health.htm

Osadchiy, V., Martin, C. R., & Mayer, E. A. (2019). The Gut-Brain Axis and the Microbiome: Mechanisms and Clinical Implications. *Clinical Gastroenterology and Hepatology: The Official Clinical Practice Journal of the American Gastroenterological Association*, *17*(2), 322–332. https://doi.org/10.1016/j.cgh.2018.10.002

Peng, L., Li, Z. R., Green, R. S., Holzman, I. R., & Lin, J. (2009). Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *The Journal of Nutrition*, *139*(9), 1619–1625. https://doi.org/10.3945/jn.109.104638

Pereira, F. C., & Berry, D. (2017). Microbial nutrient niches in the gut. *Environmental Microbiology*, *19*(4), 1366–1378. https://doi.org/10.1111/1462-2920.13659

Perez-Muñoz, M. E., Arrieta, M. C., Ramer-Tait, A. E., & Walter, J. (2017). A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. *Microbiome*, *5*(1), 48. https://doi.org/10.1186/s40168-017-0268-4

Petersen, K. J., Qualter, P., & Humphrey, N. (2019). The Application of Latent Class Analysis for Investigating Population Child Mental Health: A Systematic Review. *Frontiers in Psychology*, *10*, 1214. https://doi.org/10.3389/fpsyg.2019.01214

Peterson, D., Bonham, K. S., Rowland, S., Pattanayak, C. W., RESONANCE Consortium, & Klepac-Ceraj, V. (2021). Comparative Analysis of 16S rRNA Gene and Metagenome Sequencing in Pediatric Gut Microbiomes. *Frontiers in microbiology*, *12*, 670336. https://doi.org/10.3389/fmicb.2021.670336

Piepmeier, A. T. & Etnier, J. L. (2015). Brain-derived neurotrophic factor (BDNF) as a potential mechanism of the effects of acute exercise on cognitive performance. *Journal of Sport and Health Science*, *4*(1), 14-23. https://doi.org/10.1016/j.jshs.2014.11.001

Pignata, C., Budillon, G., Monaco, G., Nani, E., Cuomo, R., Parrilli, G., & Ciccimarra, F. (1990). Jejunal bacterial overgrowth and intestinal permeability in children with immunodeficiency syndromes. *Gut*, *31*(8), 879–882. https://doi.org/10.1136/gut.31.8.879

Piqué, N., Berlanga, M., & Miñana-Galbis, D. (2019). Health benefits of heat-killed (Tyndallized) probiotics: An overview. *International Journal of Molecular Sciences*, *20*(10), 2534. https://doi.org/10.3390/ijms20102534

Polis, S., & Fernandez, R. (2015). Impact of physical and psychological factors on health-related quality of life in adult patients with liver cirrhosis: a systematic review protocol. *JBI Database of Systematic Reviews and Implementation Reports*, *13*(1), 39–51. https://doi.org/10.11124/jbisrir-2015-1987

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., … Wang, J. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59–65. https://doi.org/10.1038/nature08821

Quercia, S., Candela, M., Giuliani, C., Turroni, S., Luiselli, D., Rampelli, S., Brigidi, P., Franceschi, C., Bacalini, M. G., Garagnani, P., & Pirazzini, C. (2014). From lifetime to evolution: Timescales of human gut microbiota adaptation. *Frontiers in Microbiology*, *5*, 587. https://doi.org/10.3389/fmicb.2014.00587

Quigley, E. M. (2013). Gut bacteria in health and disease. *Gastroenterology & hepatology*, *9*(9), 560–569.

Radjabzadeh, D., Boer, C. G., Beth, S. A., van der Wal, P., Kiefte-De Jong, J. C., Jansen, M., Konstantinov, S. R., Peppelenbosch, M. P., Hays, J. P., Jaddoe, V., Ikram, M. A., Rivadeneira, F., van Meurs, J., Uitterlinden, A. G., Medina-Gomez, C., Moll, H. A., & Kraaij, R. (2020). Diversity, compositional and functional differences between gut microbiota of children and adults. *Scientific Reports*, *10*(1), 1040. https://doi.org/10.1038/s41598-020-57734-z

Ramakrishna B. S. (2013). Role of the gut microbiota in human nutrition and metabolism. *Journal of Gastroenterology and Hepatology*, *28 Suppl 4*, 9–17. https://doi.org/10.1111/jgh.12294

Rao, M., & Gershon, M. D. (2016). The bowel and beyond: The enteric nervous system in neurological disorders. *Nature reviews. Gastroenterology & Hepatology*, *13*(9), 517–528. https://doi.org/10.1038/nrgastro.2016.107

Rao, S., Rehman, A., Yu, S., & Andino, N. M. (2018). Brain fogginess, gas and bloating: A link between SIBO, probiotics and metabolic acidosis. *Clinical and Translational Gastroenterology*, *9*(6), 162. https://doi.org/10.1038/s41424-018-0030-7

Rausch, P., Rühlemann, M. Hermes, B. M., Doms, S., Dagan, T., Dierking, K., Domin, H., Fraune, S., von Frieling, J., Hentschel, U., Heinsen, F. A., Höppner, M., Jahn, M. T., Jaspers, C., Kissoyan, K. A. B., Langfeldt, D., Rehman, A., Reusch, T. B. H., Roeder, T., … Baines, J. F. (2019). Comparative analysis of amplicon and metagenomic sequencing methods reveals key features in the evolution of animal metaorganisms. *Microbiome*, *7*, 133. https://doi.org/10.1186/s40168-019-0743-1

Rautava, S., Luoto, R., Salminen, S., & Isolauri, E. (2012). Microbial contact during pregnancy, intestinal colonization and human disease. *Nature Reviews. Gastroenterology & Hepatology*, *9*(10), 565–576. https://doi.org/10.1038/nrgastro.2012.144

Rea, K., Dinan, T. G., & Cryan, J. F. (2016). The microbiome: A key regulator of stress and neuroinflammation. *Neurobiology of Stress*, *4*, 23–33. https://doi.org/10.1016/j.ynstr.2016.03.001

Rhee, S. H., Pothoulakis, C., & Mayer, E. A. (2009). Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature reviews. Gastroenterology & Hepatology*, *6*(5), 306–314. https://doi.org/10.1038/nrgastro.2009.35

Rhodes, R. (2016). Ethical issues in microbiome research and medicine. *BMC Medicine*, *14*(1), 156. https://doi.org/10.1186/s12916-016-0702-7

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G., Gasbarrini, A., & Mele, M. C. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms*, *7*(1), 14. https://doi.org/10.3390/microorganisms7010014

Romo, J. A., & Kumamoto, C. A. (2020). On commensalism of *Candida*. *Journal of Fungi*, *6*(1), 16. https://doi.org/10.3390/jof6010016

Rossen, N. G., MacDonald, J. K., de Vries, E. M., D'Haens, G. R., de Vos, W. M., Zoetendal, E. G., & Ponsioen, C. Y. (2015). Fecal microbiota transplantation as novel therapy in gastroenterology: A systematic review. *World Journal of Gastroenterology*, *21*(17), 5359–5371. https://doi.org/10.3748/wjg.v21.i17.5359

Roubaud-Baudron, C., Ruiz, V. E., Swan, A. M., Jr, Vallance, B. A., Ozkul, C., Pei, Z., Li, J., Battaglia, T. W., Perez-Perez, G. I., & Blaser, M. J. (2019). Long-term effects of early-life antibiotic exposure on resistance to subsequent bacterial infection. *mBio*, *10*(6), e02820-19. https://doi.org/10.1128/mBio.02820-19

Rubio-Ruiz, M. E., Peredo-Escárcega, A. E., Cano-Martínez, A., & Guarner-Lans, V. (2015). An evolutionary perspective of nutrition and inflammation as mechanisms of cardiovascular disease. *International Journal of Evolutionary Biology*, *2015*, 179791. https://doi.org/10.1155/2015/179791

Rucklidge, J. J. (2013). Could yeast infections impair recovery from mental illness? A case study using micronutrients and olive leaf extract for the treatment of ADHD and depression. *Advances in Mind-Body Medicine*, *27*(3), 14–18.

Ruiz-Nunez, B., Pruimboom, L., Dijck-Brouwer, D. A., & Muskiet, F. A. (2013). Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context. *The Journal of Nutritional Biochemistry*, *24*(7), 1183–1201. https://doi.org/10.1016/j.jnutbio.2013.02.009

Salvucci E. (2019). The human-microbiome superorganism and its modulation to restore health. *International Journal of Food Sciences and Nutrition*, *70*(7), 781–795. https://doi.org/10.1080/09637486.2019.1580682

Samb-Ba, B., Mazenot, C., Gassama-Sow, A., Dubourg, G., Richet, H., Hugon, P., Lagier, J. C., Raoult, D., & Fenollar, F. (2014). MALDI-TOF identification of the human gut microbiome in people with and without diarrhea in Senegal. *PloS One*, *9*(5), e87419. https://doi.org/10.1371/journal.pone.0087419

Sarkar, A., Yoo, J. Y., Valeria Ozorio Dutra, S., Morgan, K. H., & Groer, M. (2021). The association between early-life gut microbiota and long-term health and diseases. *Journal of Clinical Medicine*, *10*(3), 459. https://doi.org/10.3390/jcm10030459

Scanlan, P. D., & Marchesi, J. R. (2008). Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces. *The ISME Journal*, *2*(12), 1183–1193. https://doi.org/10.1038/ismej.2008.76

Sarkar, A., Yoo, J. Y., Valeria Ozorio Dutra, S., Morgan, K. H., & Groer, M. (2021). The association between early-life gut microbiota and long-term health and diseases. *Journal of Clinical Medicine*, *10*(3), 459. https://doi.org/10.3390/jcm10030459

Scarpellini, E., Ianiro, G., Attili, F., Bassanelli, C., De Santis, A., & Gasbarrini, A. (2015). The human gut microbiota and virome: Potential therapeutic implications. *Digestive and Liver Disease: Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver*, *47*(12), 1007–1012. https://doi.org/10.1016/j.dld.2015.07.008

Shen, Y., Xu, J., Li, Z., Huang, Y., Yuan, Y., Wang, J., Zhang, M., Hu, S., & Liang, Y. (2018). Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: A cross-sectional study. *Schizophrenia Research*, *197*, 470–477. https://doi.org/10.1016/j.schres.2018.01.002

Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., & Burnet, P. W. (2015). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology*, *232*(10), 1793–1801. https://doi.org/10.1007/s00213-014-3810-0

Schnorr, S. L., Candela, M., Rampelli, S., Centanni, M., Consolandi, C., Basaglia, G., Turroni, S., Biagi, E., Peano, C., Severgnini, M., Fiori, J., Gotti, R., De Bellis, G., Luiselli, D., Brigidi, P., Mabulla, A., Marlowe, F., Henry, A. G., & Crittenden, A. N. (2014). Gut microbiome of the Hadza hunter-gatherers. *Nature Communications*, *5*, 3654. https://doi.org/10.1038/ncomms4654

Schwenger, K. J., Clermont-Dejean, N., & Allard, J. P. (2019). The role of the gut microbiome in chronic liver disease: The clinical evidence revised. *JHEP Reports: Innovation in Hepatology*, *1*(3), 214–226. https://doi.org/10.1016/j.jhepr.2019.04.004

Seidel, J., & Valenzano, D. R. (2018). The role of the gut microbiome during host ageing. *F1000Research*, *7*, 1086. https://doi.org/10.12688/f1000research.15121.1

Sekirov, I., Russell, S. L., Antunes, L. C., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological Reviews*, *90*(3), 859–904. https://doi.org/10.1152/physrev.00045.2009

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biology*, *14*(8), e1002533. https://doi.org/10.1371/journal.pbio.1002533

Senghor, B., Sokhna, C., Ruimy, R., & Lagier, J. C. (2018). Cut microbiota diversity according to dietary habits and geographical provenance. *Human Microbiome Journal*, *7-8*, 1-9. https://doi.org/10.1016/j.humic.2018.01.001

Severance, E. G., Gressitt, K. L., Stallings, C. R., Katsafanas, E., Schweinfurth, L. A., Savage, C. L., Adamos, M. B., Sweeney, K. M., Origoni, A. E., Khushalani, S., Leweke, F. M., Dickerson, F. B., & Yolken, R. H. (2016). Candida albicans exposures, sex specificity and cognitive deficits in schizophrenia and bipolar disorder. *NPJ Schizophrenia*, *2*, 16018. https://doi.org/10.1038/npjschz.2016.18

Shin, W. & Kim, H. J. (2018) Intestinal barrier dysfunction orchestrates the onset of inflammatory host-microbiome cross-talk in a human gut inflammation-on-a-chip. *Proceedings of the National Academy of Sciences of the United States of America*, *45*, E10539-E10547. https://doi.org/10.1073/pnas.1810819115

Shkoporov, A. N., & Hill, C. (2019). Bacteriophages of the human gut: The "Known Unknown" of the Microbiome. *Cell host & microbe*, *25*(2), 195–209. https://doi.org/10.1016/j.chom.2019.01.017

Shojaee, S., Teimouri, A., Keshavarz, H., Azami, S. J., & Nouri, S. (2018). The relation of secondary sex ratio and miscarriage history with Toxoplasma gondii infection. *BMC Infectious Diseases*, *18*(1), 307. https://doi.org/10.1186/s12879-018-3228-0

Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Frontiers in Endocrinology*, *11*, 25. https://doi.org/10.3389/fendo.2020.00025

Simon, J. C., Marchesi, J. R., Mougel, C., & Selosse, M. A. (2019). Host-microbiota interactions: From holobiont theory to analysis. *Microbiome*, *7*(1), 5. https://doi.org/10.1186/s40168-019-0619-4

Simpson, J. A., & Rholes, W. S. (2017). Adult Attachment, Stress, and Romantic Relationships. *Current Opinion in Psychology*, *13*, 19–24. https://doi.org/10.1016/j.copsyc.2016.04.006

Singh, R. K., Chang, H. W., Yan, D., Lee, K. M., Ucmak, D., Wong, K., Abrouk, M., Farahnik, B., Nakamura, M., Zhu, T. H., Bhutani, T., & Liao, W. (2017). Influence of diet on the gut microbiome and implications for human health. *Journal of Translational Medicine*, *15*(1), 73. https://doi.org/10.1186/s12967-017-1175-y

Singhal, N., Kumar, M., Kanaujia, P. K., & Virdi, J. S. (2015). MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology*, *6*, 791. https://doi.org/10.3389/fmicb.2015.00791

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., Glickman, J. N., & Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science (New York, N.Y.)*, *341*(6145), 569–573. https://doi.org/10.1126/science.1241165

Smits, S. A., Leach, J., Sonnenburg, E. D., Gonzalez, C. G., Lichtman, J. S., Reid, G., Knight, R., Manjurano, A., Changalucha, J., Elias, J. E., Dominguez-Bello, M. G., & Sonnenburg, J. L. (2017). Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science*, *357*(6353), 802–806. https://doi.org/10.1126/science.aan4834

So, S. Y., & Savidge, T. C. (2021). Sex-bias in irritable bowel syndrome: Linking steroids to the gut-brain axis. *Frontiers in endocrinology*, *12*, 684096. https://doi.org/10.3389/fendo.2021.684096

Sonnenburg, E. D., Smits, S. A., Tikhonov, M., Higginbottom, S. K., Wingreen, N. S., & Sonnenburg, J. L. (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature*, *529*(7585), 212–215. https://doi.org/10.1038/nature16504

Soto, M., Herzog, C., Pacheco, J. A., Fujisaka, S., Bullock, K., Clish, C. B., & Kahn, C. R. (2018). Gut microbiota modulate neurobehavior through changes in brain insulin sensitivity and metabolism. *Molecular Psychiatry*, *23*(12), 2287–2301. https://doi.org/10.1038/s41380-018-0086-5

Statovci, D., Aguilera, M., MacSharry, J., & Melgar, S. (2017). The impact of Western diet and nutrients on the microbiota and immune response at mucosal interfaces. *Frontiers in Immunology*, *8*, 838. https://doi.org/10.3389/fimmu.2017.00838

Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J. A., & Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, Behavior, and Immunity*, *48*, 258–264. https://doi.org/10.1016/j.bbi.2015.04.003

Stein, C. M., Morris, N. J., Hall, N. B., & Nock, N. L. (2017). Structural equation modeling. *Methods in Molecular Biology*, *1666*, 557–580. https://doi.org/10.1007/978-1-4939-7274-6_28

Stilling, R. M., van de Wouw, M., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2016). The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis?. *Neurochemistry international*, *99*, 110–132. https://doi.org/10.1016/j.neuint.2016.06.011

Stinson, L. F., Boyce, M. C., Payne, M. S., & Keelan, J. A. (2019). The not-so-sterile womb: Evidence that the human fetus is exposed to bacteria prior to birth. *Frontiers in Microbiology*, *10*, 1124. https://doi.org/10.3389/fmicb.2019.01124

Strati, F., Cavalieri, D., Albanese, D., De Felice, C., Donati, C., Hayek, J., Jousson, O., Leoncini, S., Renzi, D., Calabrò, A., & De Filippo, C. (2017). New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome*, *5*(1), 24. https://doi.org/10.1186/s40168-017-0242-1

Straub, R. H. (2007). The complex role of estrogens in inflammation. *Endocrine Reviews*, *28*(5), 521–574. https://doi.org/10.1210/er.2007-0001

Strejcek, M., Smrhova, T., Junkova, P., & Uhlik, O. (2018). Whole-cell MALDI-TOF MS versus 16S rRNA gene analysis for identification and dereplication of recurrent bacterial isolates. *Frontiers in Microbiology*, *9*, 1294. https://doi.org/10.3389/fmicb.2018.01294

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., Kubo, C., & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of Physiology*, *558*(1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Sugahara, H., Yao, R., Odamaki, T., & Xiao, J. Z. (2017). Differences between live and heat-killed bifidobacteria in the regulation of immune function and the intestinal environment. *Beneficial Microbes*, *8*(3), 463–472. https://doi.org/10.3920/BM2016.0158

Sun, Y. Y., Li, M., Li, Y. Y., Li, L. X., Zhai, W. Z., Wang, P., Yang, X. X., Gu, X., Song, L. J., Li, Z., Zuo, X. L., & Li, Y. Q. (2018). The effect of Clostridium butyricum on symptoms and fecal microbiota in diarrhea-dominant irritable bowel syndrome: A randomized, double-blind, placebo-controlled trial. *Scientific Reports*, *8*(1), 2964. https://doi.org/10.1038/s41598-018-21241-z

Sung, J., Hale, V., Merkel, A. C., Kim, P. J., & Chia, N. (2016). Metabolic modeling with Big Data and the gut microbiome. *Applied & Translational Genomics*, *10*, 10–15. https://doi.org/10.1016/j.atg.2016.02.001

Taber, K. S. (2018). The use of Cronbach's alpha when developing and reporting research instruments in science education. *Research in Science Education*, *48*, 1273-1296. https://doi.org/10.1007/s11165-016-9602-2

Talari, K., & Goyal, M. (2020). Retrospective studies - utility and caveats. *The Journal of the Royal College of Physicians of Edinburgh*, *50*(4), 398–402. https://doi.org/10.4997/JRCPE.2020.409

Tang, Q., Jin, G., Wang, G., Liu, T., Liu, X., Wang, B., & Cao, H. (2020). Current sampling methods for gut microbiota: A call for more precise devices. *Frontiers in Cellular and Infection Microbiology*, *10*, 151. https://doi.org/10.3389/fcimb.2020.00151

Taylor, A. M., Thompson, S. V., Edwards, C. G., Musaad, S., Khan, N. A., & Holscher, H. D. (2019). Associations among diet, the gastrointestinal microbiota, and negative emotional states in adults. *Nutritional Neuroscience*, *23*(12), 983–992. https://doi.org/10.1080/1028415X.2019.1582578

Teo, A. R., Choi, H., & Valenstein, M. (2013). Social relationships and depression: ten-year follow-up from a nationally representative study. *PloS One*, *8*(4), e62396. https://doi.org/10.1371/journal.pone.0062396

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C., Cryan, J. F., Gilbert, S. F., Goodnight, C. J., Lloyd, E. A., Sapp, J., Vandenkoornhuyse, P., Zilber-Rosenberg, I., Rosenberg, E., & Bordenstein, S. R. (2016). Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes. *mSystems*, *1*(2), e00028-16. https://doi.org/10.1128/mSystems.00028-16

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *The Biochemical Journal*, *474*(11), 1823–1836. https://doi.org/10.1042/BCJ20160510

Trinh, P., Zaneveld, J. R., Safranek, S., & Rabinowitz, P. M. (2018). One health relationships between human, animal, and environmental microbiomes: A mini-review. *Frontiers in Public Health*, *6*, 235. https://doi.org/10.3389/fpubh.2018.00235

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., Guyonnet, D., Legrain-Raspaud, S., Trotin, B., Naliboff, B., & Mayer, E. A. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394–1401.e14014. https://doi.org/10.1053/j.gastro.2013.02.043

Tun, H. M., Konya, T., Takaro, T. K., Brook, J. R., Chari, R., Field, C. J., Guttman, D. S., Becker, A. B., Mandhane, P. J., Turvey, S. E., Subbarao, P., Sears, M. R., Scott, J. A., Kozyrskyj, A. L., & CHILD Study Investigators. (2017). Exposure to household furry pets influences the gut microbiota of infant at 3-4 months following various birth scenarios. *Microbiome*, *5*(1), 40. https://doi.org/10.1186/s40168-017-0254-x

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. A., Affourtit, J. P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R., & Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228), 480–484. https://doi.org/10.1038/nature07540

Turroni, F., Milani, C., Duranti, S., Lugli, G. A., Bernasconi, S., Margolles, A., Di Pierro, F., van Sinderen, D., & Ventura, M. (2020). The infant gut microbiome as a microbial organ influencing host well-being. *Italian Journal of Pediatrics*, *46*(1), 16. https://doi.org/10.1186/s13052-020-0781-0

Umberson, D., & Montez, J. K. (2010). Social relationships and health: A flashpoint for health policy. *Journal of Health and Social Behavior*, *51*, 54–66. https://doi.org/10.1177/0022146510383501

Ursell, L. K., Metcalf, J. L., Parfrey, L. W., & Knight, R. (2012). Defining the human microbiome. *Nutrition Reviews*, *70 Suppl 1*(Suppl 1), S38–S44. https://doi.org/10.1111/j.1753-4887.2012.00493.x

Vagnerová, K., Vodička, M., Hermanová, P., Ergang, P., Šrůtková, D., Klusoňová, P., Balounová, K., Hudcovic, T., & Pácha, J. (2019). Interactions between gut microbiota and acute restraint stress in peripheral structures of the hypothalamic-pituitary-adrenal axis and the intestine of male mice. *Frontiers in Immunology*, *10*, 2655. https://doi.org/10.3389/fimmu.2019.02655

Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *BMJ*, *361*, k2179. https://doi.org/10.1136/bmj.k2179

van de Guchte, M., Blottière, H. M., & Doré, J. (2018). Humans as holobionts: Implications for prevention and therapy. *Microbiome*, *6*(1), 81. https://doi.org/10.1186/s40168-018-0466-8

Vandenbroucke, J. P., von Elm, E., Altman, D. G., Gøtzsche, P. C., Mulrow, C. D., Pocock, S. J., Poole, C., Schlesselman, J. J., Egger, M., & STROBE Initiative (2007). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Epidemiology (Cambridge, Mass.)*, *18*(6), 805–835. https://doi.org/10.1097/EDE.0b013e3181577511

van de Wouw, M., Boehme, M., Lyte, J. M., Wiley, N., Strain, C., O'Sullivan, O., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2018). Short-chain fatty acids: Microbial metabolites that alleviate stress-induced brain-gut axis alterations. *The Journal of Physiology*, *596*(20), 4923–4944. https://doi.org/10.1113/JP276431

van Doorn, J., Ly, A., Marsman, M., & Wagenmakers, E. J. (2018) Bayesian inference for Kendall's rank correlation coefficient. *The American Statistician*, *72*(4), 303-308. https://doi.org/10.1080/00031305.2016.1264998

van Lunzen, J., & Altfeld, M. (2014). Sex differences in infectious diseases-common but neglected. *The Journal of Infectious Diseases*, *209 Suppl 3*, S79–S80. https://doi.org/10.1093/infdis/jiu159

Vemuri, R., Gundamaraju, R., Shastri, M. D., Shukla, S. D., Kalpurath, K., Ball, M., Tristram, S., Shankar, E. M., Ahuja, K., & Eri, R. (2018). Gut microbial changes, interactions, and their implications on human lifecycle: An ageing perspective. *BioMed Research International*, *2018*, 4178607. https://doi.org/10.1155/2018/4178607

Vemuri, R., Sylvia, K. E., Klein, S. L., Forster, S. C., Plebanski, M., Eri, R., & Flanagan, K. L. (2019). The microgenderome revealed: Sex differences in bidirectional interactions between the microbiota, hormones, immunity and disease susceptibility. *Seminars in Immunopathology*, *41*(2), 265–275. https://doi.org/10.1007/s00281-018-0716-7

Venegas, D. P., De la Fuente, M. K., Landskron, G., González, M. J., Quera, R., Dijkstra, G., Harmsen, H., Faber, K. N., & Hermoso, M. A. (2019). Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Frontiers in Immunology*, *10*, 277. https://doi.org/10.3389/fimmu.2019.00277

Vich Vila, A., Collij, V., Sanna, S., Sinha, T., Imhann, F., Bourgonje, A. R., Mujagic, Z., Jonkers, D. M. A. E., Masclee, A. A. M., Fu, J., Kurilshikov, A., Wijmenga, C., Zhernakova, A., & Weersma, R. K. (2020). Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nature Communications*, *11*, 362. https://doi.org/10.1038/s41467-019-14177-z

Vinograd, M., Williams, A., Sun, M., Bobova, L., Wolitzky-Taylor, K. B., Vrshek-Schallhorn, S., Mineka, S., Zinbarg, R. E., & Craske, M. G. (2020). Neuroticism and interpretive bias as risk factors for

anxiety and depression. *Clinical psychological science: A journal of the Association for Psychological Science*, *8*(4), 641–656. https://doi.org/10.1177/2167702620906145

Vogelzangs, N., Beekman, A. T., de Jonge, P., & Penninx, B. W. (2013). Anxiety disorders and inflammation in a large adult cohort. *Translational Psychiatry*, *3*(4), e249. https://doi.org/10.1038/tp.2013.27

Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., Carlsson, C. M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B. B., & Rey, F. E. (2017). Gut microbiome alterations in Alzheimer's disease. *Scientific Reports*, *7*(1), 13537. https://doi.org/10.1038/s41598-017-13601-y

Waimin, J. F., Nejati, S., Jiang, H., Qui, J., Wang, J., Verma, M. S., & Rahimi, R. (2020). Smart capsule for non-invasive sampling and studying of the gastrointestinal microbiome. *RSC Advances*, *10,* 16313. https://doi.org/10.1039/C9RA10986B

Walker, A. J., Kim, Y., Price, J. B., Kale, R. P., McGillivray, J. A., Berk, M., & Tye, S. J. (2014). Stress, inflammation, and cellular vulnerability during early stages of affective disorders: Biomarker strategies and opportunities for prevention and intervention. *Frontiers in Psychiatry*, *5*, 34. https://doi.org/10.3389/fpsyt.2014.00034

Wallis, A., Ball, M., Butt, H., Lewis, D. P., McKechnie, S., Paull, P., Jaa-Kwee, A., & Bruck, D. (2018). Open-label pilot for treatment targeting gut dysbiosis in myalgic encephalomyelitis/chronic fatigue syndrome: neuropsychological symptoms and sex comparisons. *Journal of Translational Medicine*, *16*(1), 24. https://doi.org/10.1186/s12967-018-1392-z

Wallis, A., Butt, H., Ball, M., Lewis, D. P., & Bruck, D. (2016). Support for the microgenderome: Associations in a human clinical population. *Scientific Reports, 6*, 19171. https://doi.org/10.1038/srep19171

Walsh, J., Griffin, B. T., Clarke, G., & Hyland, N. P. (2018). Drug-gut microbiota interactions: Implications for neuropharmacology. *British Journal of Pharmacology*, *175*(24), 4415–4429. https://doi.org/10.1111/bph.14366

Wang, J., & Lin, L. (2019). Early-life gut microbiota development from maternal vertical transmission. *Gynecology and Obstetrics Clinical Medicine*, *1*(2), 79-82. https://doi.org/10.1016/j.gocm.2021.03.008

Wang, M., Monaco, M. H., & Donovan, S. M. (2016). Impact of early gut microbiota on immune and metabolic development and function. *Seminars in Fetal & Neonatal Medicine*, *21*(6), 380–387. https://doi.org/10.1016/j.siny.2016.04.004

Wang, Y., & Kasper, L. H. (2014). The role of microbiome in central nervous system disorders. *Brain, Behavior, and Immunity*, *38*, 1–12. https://doi.org/10.1016/j.bbi.2013.12.015

Wang, Y., Wang, Z., Wang, Y., Li, F., Jia, J., Song, X., Qin, S., Wang, R., Jin, F., Kitazato, K., & Wang, Y. (2018). The Gut-Microglia Connection: Implications for Central Nervous System Diseases. *Frontiers in immunology*, *9*, 2325. https://doi.org/10.3389/fimmu.2018.02325

Wauthia, E., Lefebvre, L., Huet, K., Blekic, W., El Bouragui, K., & Rossignol, M. (2019). Examining the hierarchical influences of the big-five dimensions and anxiety sensitivity on anxiety symptoms in children. *Frontiers in Psychology*, *10*, 1185. https://doi.org/10.3389/fpsyg.2019.01185

Weersma, R. K., Zhernakova, A., & Fu, J. (2020). Interaction between drugs and the gut microbiome. *Gut*, *69*(8), 1510–1519. https://doi.org/10.1136/gutjnl-2019-320204

Weir, C. B. & Le, J. K. (2020). Metronidazole. In: StatPearls (internet). StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539728/

Whitlock, F. A. (1961). Some psychiatric consequences of gastreotomy. *British Medical Journal*, *1*(5239), 1560–1564. https://doi.org/10.1136/bmj.1.5239.1560

Wiertsema, S. P., van Bergenhenegouwen, J., Garssen, J., & Knippels, L. M. J. (2021) The interplay between the gut microbiome and the immune system in the context of infectious diseases throughout life and the role of nutrition in optimizing treatment strategies, *Nutrients*, *13*(3), 886. https://doi.org/10.3390/nu13030886

Winter, G., Hart, R. A., Charlesworth, R., & Sharpley, C. F. (2018). Gut microbiome and depression: What we know and what we need to know. *Reviews in the Neurosciences*, *29*(6), 629–643. https://doi.org/10.1515/revneuro-2017-0072

World Health Organisation. (2017). *Depression and Other Common Mental Disorders: Global Health Estimates.* Retrieved July 9, 2021 from https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf

World Health Organisation. (2020). *The top 10 causes of death*. Retrieved February 20, 2021 from https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D., & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, *334*(6052), 105–108. https://doi.org/10.1126/science.1208344

Wylezich, C., Belka, A., Hanke, D., Beer, M., Blome, S., & Höper, D. (2019). Metagenomics for broad and improved parasite detection: a proof-of-concept study using swine faecal

samples. *International Journal for Parasitology*, *49*(10), 769–777. https://doi.org/10.1016/j.ijpara.2019.04.007

Yamamura, S., Morishima, H., Kumano-go, T., Suganuma, N., Matsumoto, H., Adachi, H., Sigedo, Y., Mikami, A., Kai, T., Masuyama, A., Takano, T., Sugita, Y., & Takeda, M. (2009). The effect of Lactobacillus helveticus fermented milk on sleep and health perception in elderly subjects. *European Journal of Clinical Nutrition*, *63*(1), 100–105. https://doi.org/10.1038/sj.ejcn.1602898

Yang, Y. C., Boen, C., Gerken, K., Li, T., Schorpp, K., & Harris, K. M. (2016). Social relationships and physiological determinants of longevity across the human life span. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(3), 578–583. https://doi.org/10.1073/pnas.1511085112

Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., Nagler, C. R., Ismagilov, R. F., Mazmanian, S. K., & Hsiao, E. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, *161*(2), 264–276. https://doi.org/10.1016/j.cell.2015.02.047

Yolken, R., Torrey, E. F., & Dickerson, F. (2017). Evidence of increased exposure to Toxoplasma gondii in individuals with recent onset psychosis but not with established schizophrenia. *PLoS Neglected Tropical Diseases*, *11*(11), e0006040. https://doi.org/10.1371/journal.pntd.0006040

Yoon, K., & Kim, N. (2021). Roles of sex hormones and gender in the gut microbiota. *Journal of Neurogastroenterology and Motility*, *27*(3), 314–325. https://doi.org/10.5056/jnm20208

Yoshii, K., Hosomi, K., Sawane, K., & Kunisawa, J. (2019). Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. *Frontiers in Nutrition*, *6*, 48. https://doi.org/10.3389/fnut.2019.00048

Young, M. S., & Schinka, J. A. (2001). Research validity scales for the NEO-PI-R: Additional evidence for reliability and validity. *Journal of Personality Assessment*, *76*(3), 412–420. https://doi.org/10.1207/S15327752JPA7603_04

Yuan, X., Chen, R., Zhang, Y., Lin, X., & Yang, X. (2020). Sexual dimorphism of gut microbiota at different pubertal status. *Microbial Cell Factories*, *19*(1), 152. https://doi.org/10.1186/s12934-020-01412-2

Yurkovetskiy, L., Burrows, M., Khan, A. A., Graham, L., Volchkov, P., Becker, L., Antonopoulos, D., Umesaki, Y., & Chervonsky, A. V. (2013). Gender bias in autoimmunity is influenced by microbiota. *Immunity*, *39*(2), 400–412. https://doi.org/10.1016/j.immuni.2013.08.013

Zampieri, A., Babbucci, M., Carraro, L., Milan, M., Fasolato, L., & Cardazzo, B. (2021). Combining culture-dependent and culture-independent methods: New methodology insight on the Vibrio community of Ruditapes philippinarum. *Foods*, *10*(6), 1271, https://doi.org/10.3390/foods10061271

Zazula, R., Husain, M. I., Mohebbi, M., Walker, A. J., Chaudhry, I. B., Khoso, A. B., Ashton, M. M., Agustini, B., Husain, N., Deakin, J., Young, A. H., Berk, M., Kanchanatawan, B., Ng, C. H., Maes, M., Berk, L., Singh, A. B., Malhi, G. S., & Dean, O. M. (2021). Minocycline as adjunctive treatment for major depressive disorder: Pooled data from two randomized controlled trials. *The Australian and New Zealand journal of Psychiatry*, *55*(8), 784–798. https://doi.org/10.1177/0004867420965697

Zha, Y., Chong, H., & Ning, K. (2021). Microbiome sample comparison and search: From pair-wise calculations to model-based matching. *Frontiers in Microbiology*, *12*, 642439. https://doi.org/10.3389/fmicb.2021.642439

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Research*, *30*(6), 492–506. https://doi.org/10.1038/s41422-020-0332-7

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N. D., Licinio, J., Wei, H., & Xie, P. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular Psychiatry*, *21*(6), 786–796. https://doi.org/10.1038/mp.2016.44

Zhu, L., Luo, F., Hu, W., Han, Y., Wang, Y., Zheng, H., Guo, X., & Qin, J. (2018). Bacterial communities in the womb during healthy pregnancy. *Frontiers in Microbiology*, *9*, 2163. https://doi.org/10.3389/fmicb.2018.02163

Zhu, S., Jiang, Y., Xu, K., Cui, M., Ye, W., Zhao, G., Jin, L., & Chen, X. (2020). The progress of gut microbiome research related to brain disorders. *Journal of Neuroinflammation*, *17*(1), 25. https://doi.org/10.1186/s12974-020-1705-z

Zinöcker, M. K., & Lindseth, I. A. (2018). The Western diet-microbiome-host interaction and its role in metabolic disease. *Nutrients*, *10*(3), 365. https://doi.org/10.3390/nu10030365

Appendix A

Bioscreen Patient Questionnaire (BPQ)

Severity is rated from 4 indicating extreme to 0 meaning none at all frequency is rated from 4 indicating constant to 1 meaning rarely and 0 indicating none at all.	*Severity over the past 7 days*	*Frequency over the past year*
1. Headaches	0 1 2 3 4	0 1 2 3 4
2. Sinusitis or nasal congestion	0 1 2 3 4	0 1 2 3 4
3. Repeated unpleasant thoughts	0 1 2 3 4	0 1 2 3 4
4. Faintness or dizziness	0 1 2 3 4	0 1 2 3 4
5. Loss of libido or sexual interest	0 1 2 3 4	0 1 2 3 4
6. Night sweats, unusual sweating while asleep	0 1 2 3 4	0 1 2 3 4
7. Migraine headaches	0 1 2 3 4	0 1 2 3 4
8. Unusual muscle twitches	0 1 2 3 4	0 1 2 3 4
9. Trouble remembering things	0 1 2 3 4	0 1 2 3 4
10. Frequent muscle cramps	0 1 2 3 4	0 1 2 3 4
11. Grinding or clenching your teeth	0 1 2 3 4	0 1 2 3 4
12. Chest or heart pain	0 1 2 3 4	0 1 2 3 4
13. Face pain or tenderness	0 1 2 3 4	0 1 2 3 4
14. Feeling low in energy or fatigued	0 1 2 3 4	0 1 2 3 4
15. Neck pain or tenderness	0 1 2 3 4	0 1 2 3 4
16. Shoulder pain or tenderness	0 1 2 3 4	0 1 2 3 4
17. Allergies, intolerance or reactivity to food	0 1 2 3 4	0 1 2 3 4
18. Arthritis	0 1 2 3 4	0 1 2 3 4
19. Poor appetite	0 1 2 3 4	0 1 2 3 4
20. Crying easily over your problems	0 1 2 3 4	0 1 2 3 4
21. Arm pain or tenderness	0 1 2 3 4	0 1 2 3 4
22. Leg pain or tenderness	0 1 2 3 4	0 1 2 3 4
23. Abdominal pain or tenderness	0 1 2 3 4	0 1 2 3 4
24. Stiff or painful joints first thing in the morning	0 1 2 3 4	0 1 2 3 4
25. Joints that hurt when you move	0 1 2 3 4	0 1 2 3 4
26. Locking or clicking of jaw	0 1 2 3 4	0 1 2 3 4
27. Pain or tenderness in your lower back	0 1 2 3 4	0 1 2 3 4
28. Feeling that your problems are disrupting your life	0 1 2 3 4	0 1 2 3 4
29. Tinnitus or noise in the ear	0 1 2 3 4	0 1 2 3 4
30. Feeling blue as a results of your problem	0 1 2 3 4	0 1 2 3 4
31. Sore throat	0 1 2 3 4	0 1 2 3 4
32. Feeling no interest in things	0 1 2 3 4	0 1 2 3 4
33. Photophobia or dislike of strong light	0 1 2 3 4	0 1 2 3 4
34. Unrefreshed or prolonged sleep	0 1 2 3 4	0 1 2 3 4
35. Stress from financial problems	0 1 2 3 4	0 1 2 3 4

36. Feeling that others are unsympathetic to your problems	0 1 2 3 4	0 1 2 3 4
37. Unexplained diarrhoea	0 1 2 3 4	0 1 2 3 4
38. Having to do things slowly to ensure they are correct	0 1 2 3 4	0 1 2 3 4
39. Heart pounding	0 1 2 3 4	0 1 2 3 4
40. Nausea or upset stomach	0 1 2 3 4	0 1 2 3 4
41. Constipation	0 1 2 3 4	0 1 2 3 4
42. Muscle soreness or stiffness	0 1 2 3 4	0 1 2 3 4
43. Frequent urination	0 1 2 3 4	0 1 2 3 4
44. Trouble falling asleep	0 1 2 3 4	0 1 2 3 4
45. Persistent cough	0 1 2 3 4	0 1 2 3 4
46. Difficulty in making decisions	0 1 2 3 4	0 1 2 3 4
47. Ovulation or menstruation pain	0 1 2 3 4	0 1 2 3 4
48. Breathlessness or chest pain upon exertion	0 1 2 3 4	0 1 2 3 4
49. Hot and cold spells or recurrent feverishness	0 1 2 3 4	0 1 2 3 4
50. Avoiding certain activities due to physical problems	0 1 2 3 4	0 1 2 3 4
51. Mind going blank	0 1 2 3 4	0 1 2 3 4
52. Loss of feeling, tingling or numbness of the skin	0 1 2 3 4	0 1 2 3 4
53. Sore or swollen lymph glands in the neck	0 1 2 3 4	0 1 2 3 4
54. Feelings of hopelessness about the future	0 1 2 3 4	0 1 2 3 4
55. Trouble concentrating	0 1 2 3 4	0 1 2 3 4
56. Muscle weakness or feeling of weakness in the body	0 1 2 3 4	0 1 2 3 4
57. Burning or uncomfortable urination	0 1 2 3 4	0 1 2 3 4
58. Unusual post exertion/exercise fatigue	0 1 2 3 4	0 1 2 3 4
59. Sore or swollen lymph glands	0 1 2 3 4	0 1 2 3 4
60. Orchialgia or testicular pain	0 1 2 3 4	0 1 2 3 4
61. Reactivity to smells or chemicals	0 1 2 3 4	0 1 2 3 4
62. Forgetfulness	0 1 2 3 4	0 1 2 3 4
63. Urgent urination	0 1 2 3 4	0 1 2 3 4
64. Trouble waking up in the morning	0 1 2 3 4	0 1 2 3 4
65. Sore or swollen lymph glands in the groin	0 1 2 3 4	0 1 2 3 4
66. Restless or disturbed sleep	0 1 2 3 4	0 1 2 3 4
67. Vaginal irritation or discomfort	0 1 2 3 4	0 1 2 3 4
68. Hypersensitive skin	0 1 2 3 4	0 1 2 3 4
69. Feelings of mental tiredness or fatigue	0 1 2 3 4	0 1 2 3 4
70. Difficulty using words or language	0 1 2 3 4	0 1 2 3 4
71. Trouble focusing your eyes	0 1 2 3 4	0 1 2 3 4
72. Spells of panic related to your problems	0 1 2 3 4	0 1 2 3 4
73. Sciatica or numbness/tingling down the back of the leg	0 1 2 3 4	0 1 2 3 4

74. Frequently getting into arguments	0 1 2 3 4	0 1 2 3 4
75. Cold hands or feet	0 1 2 3 4	0 1 2 3 4
76. Recurrent mouth ulcers	0 1 2 3 4	0 1 2 3 4
77. Symptoms of irritable bowel	0 1 2 3 4	0 1 2 3 4
78. Mental confusion or losing your train of thought	0 1 2 3 4	0 1 2 3 4
79. Stressful events in your life related to your problems	0 1 2 3 4	0 1 2 3 4
80. Dermatitis	0 1 2 3 4	0 1 2 3 4
81. Stress over family problems	0 1 2 3 4	0 1 2 3 4
82. Gastric reflux or heartburn	0 1 2 3 4	0 1 2 3 4
83. Cravings for certain foods	0 1 2 3 4	0 1 2 3 4
84. High blood pressure	0 1 2 3 4	0 1 2 3 4
85. Low blood pressure	0 1 2 3 4	0 1 2 3 4
86. Stress from work problems	0 1 2 3 4	0 1 2 3 4
87. Feeling anxious	0 1 2 3 4	0 1 2 3 4
88. Feelings of guilt	0 1 2 3 4	0 1 2 3 4

Appendix B

Please note, the following is an excerpt from unpublished work undertaken by the broader research team. This information relates to the validity of the BPQ.

3.2 Analysis Part 1.1

With alpha set at 0.05, a split-half reliability measure using a bivariate pearson's correlation analysis was run to further test internal consistency of five of the seven factors identified on the BPQ (cognitive, sleep, depression, somatic-anxiety and stress). Bivariate Pearson's correlation established that there was a strong, statistically significant positive linear relationship between cognitive (BPQ) and CFQ for both halves ($r(90) = .607$, $p < .05$; $r(91) = .651$, $p < .05$); between sleep (BPQ) and PSQI for both halves ($r(90) = .650$, $p < .05$; $r(91) = .723$, $p < .05$); between depression (BPQ) and DASS21 depression for both halves ($r(90) = .774$, $p < .05$; $r(91) = .783$, $p < .05$); between somatic-anxiety (BPQ) and DASS21 anxiety for both halves ($r(90) = .645$, $p < .05$; $r(91) = .675$, $p < .05$); and between stress (BPQ) and DASS21 stress for when splitting the data into two halves ($r(90) = .465$, $p < .05$; $r(91) = .649$, $p < .05$).

Table 4 (below) illustrates the Pearson's Correlation Coefficient values for each of the five factors of the BPQ (cognitive, sleep, depression, somatic-anxiety, stress) in association to the five scales used for comparison that measure matching constructs (CFQ, PSQI, DASS21 depression, DAS21 anxiety, DASS21 stress).

Table 4

Split-Half Reliability Measure Using Pearson's Correlation Coefficient (N1=90, N2 =91)

	CFQ	PSQI	DASS21 Depression	DASS21 Anxiety	DASS21 Stress
Cognitive (BPQ)					
N1	*.607**				
N2	*.651**				
Sleep (BPQ)					
N1		*.650**			
N2		*.723**			
Depression (BPQ)					
N1			*.774**		
N2			*.783**		
Anxiety (BPQ)					
N1				*.645**	
N2				*.675**	
Stress (BPQ)					
N1					*.465**
N2					*.649**

Note. * $p < .05$.

As illustrated in Table 4, the Pearson's Correlation Coefficient value remained statically significant for both halves of the data, suggesting a strong linear relationship to exist among all five factors tested against their scales of comparison. However, stress on the BPQ did vary in the first half of the data tested (N1), showing to have a statistically significant moderate positive relationship of r=.465 to the DASS21 stress scale.

With alpha set at 0.05, an additional series of Pearson's Correlations were run to test the convergent and discriminant validity of five of the seven factors identified on the BPQ (cognitive, sleep, depression, somatic-anxiety and stress).

Bivariate Pearson's correlation established strong, statistically significant positive linear relationship to exist between the cognitive factor (BPQ) and the CFQ ($r(181) = .630$, $p < .05$); between somatic-anxiety (BPQ) and DASS21 anxiety ($r(181) = .667$, $p < .05$); between sleep (BPQ) and PSQI ($r(181) = .683$, $p < .05$); between stress (BPQ) and DASS21 stress ($r(181) = .555$, $p < .05$); and between depression (BPQ) and DASS21 depression ($r(181) = .774$, $p < .05$).

Bivariate Pearson's correlation established moderate, statistically significant positive linear relationship to exist between the cognitive factor (BPQ) and the PSQI ($r(181) = .475$, $p < .05$); between somatic-anxiety (BPQ) and DASS21 stress ($r(181) = .475$, $p < .05$); between sleep (BPQ) and DASS21 anxiety ($r(181) = .438$, $p < .05$); between stress (BPQ) and the CFQ ($r(181) = .432$, $p < .05$); and between depression (BPQ) and the CFQ ($r(181) = .461$, $p < .05$).

Figures 1.1 through to 5.2 (below) illustrate the convergent and divergent validity of all five factors tested on the BPQ, using a series of scatterplots to display their strength and relationships to both similar and distinct other scales of measurement.

Figure 1.1

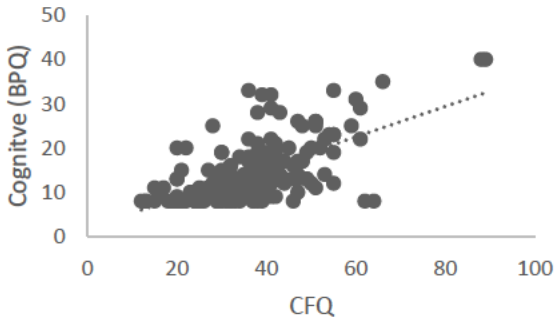


Figure 1.2

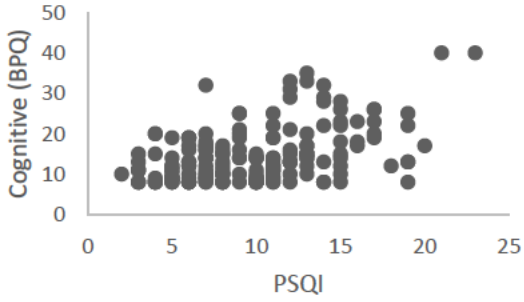


Figure 1.1. Evidence supporting the convergence validity of the BPQ cognitive factor. The BPQ cognitive factor is strongly correlated with another measure of cognition, the CFQ

(Pearson's r =.630), providing evidence for convergent validity. *Figure 1.2.* Evidence supporting the discriminant validity of the BPQ cognitive factor. As expected, the BPQ cognitive factor is only moderately correlated with the PSQI, (Pearson's r =.475) providing evidence for discriminant validity.

Figure 2.1

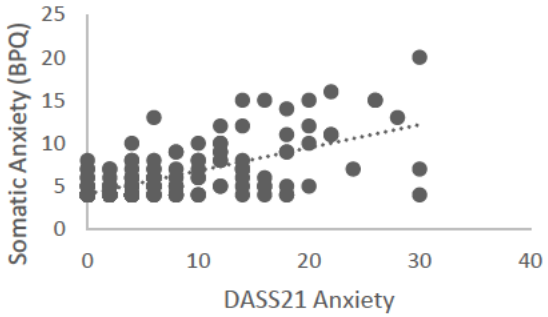


Figure 2.2

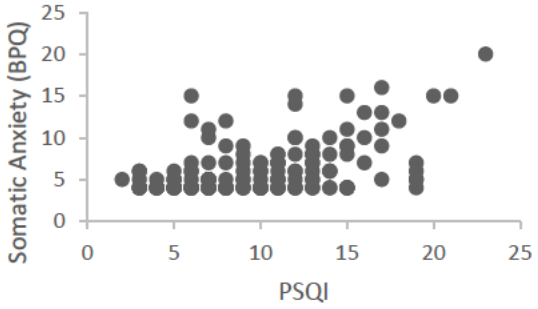


Figure 2.1. Evidence supporting the convergence validity of the BPQ somatic-anxiety factor. The BPQ somatic-anxiety factor is strongly correlated with another measure of anxiety, the DASS21 anxiety scale (Pearson's r =.667), providing evidence for convergent validity. *Figure 2.2.* Evidence supporting the discriminant validity of the BPQ somatic-anxiety factor. As expected, the BPQ somatic-anxiety factor is only moderately correlated with the PSQI, (Pearson's r =.483) providing evidence for discriminant validity.

Figure 3.1

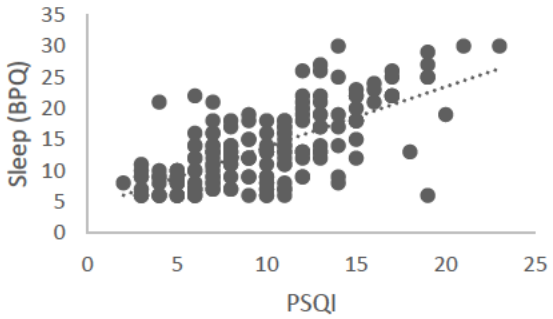


Figure 3.2

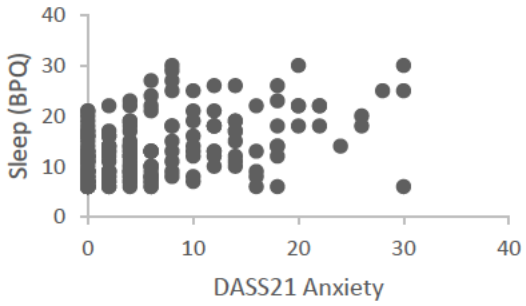
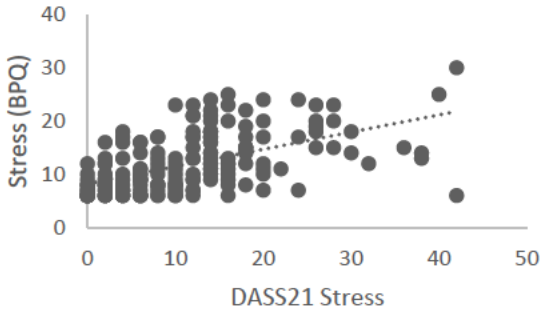


Figure 3.1. Evidence supporting the convergence validity of the BPQ sleep factor. The BPQ sleep factor is strongly correlated with another measure of sleep, the PSQI (Pearson's r =.683), providing evidence for convergent validity. *Figure 3.2.* Evidence supporting the discriminant validity of the BPQ sleep factor. As expected, the BPQ sleep factor is only moderately correlated with the DASS21 anxiety scale, (Pearson's r =.438) providing evidence for discriminant validity.

Figure 4.1

Figure 4.2



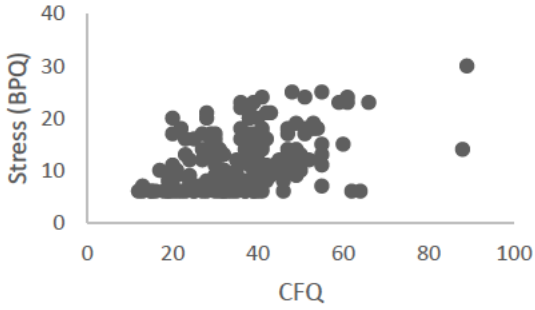


Figure 4.1. Evidence supporting the convergence validity of the BPQ stress factor. The BPQ stress factor is strongly correlated with another measure of stress, the DASS21 stress scale (Pearson's *r* =.555), providing evidence for convergent validity. *Figure 4.2.* Evidence supporting the discriminant validity of the BPQ stress factor. As expected, the BPQ stress factor is only moderately correlated with the CFQ, (Pearson's *r* =.432) providing evidence for discriminant validity.

Figure 5.1

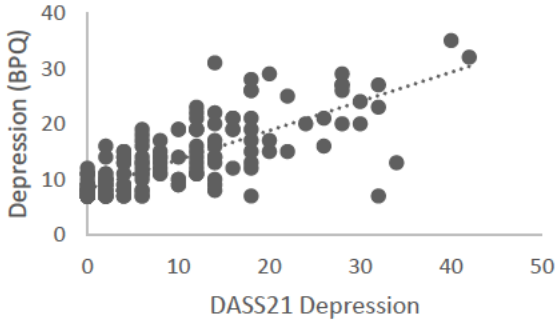


Figure 5.2

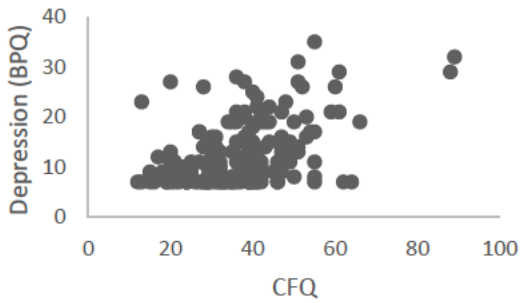


Figure 5.1. Evidence supporting the convergence validity of the BPQ depression factor. The BPQ depression factor is strongly correlated with another measure of depression, the DASS21 depression scale (Pearson's *r* =.774), providing evidence for convergent validity. *Figure 3.2.* Evidence supporting the discriminant validity of the BPQ depression factor. As expected, the BPQ depression factor is only moderately correlated with the CFQ, (Pearson's *r* =.461) providing evidence for discriminant validity.

As illustrated in figure 1.1 through to figure 5.2, factors on the BPQ correlated more statistically stronger with measures of the same construct, and showed less strong, moderate correlations with measures of distinct other constructs, providing evidence for both convergent and discriminant validity.

Appendix C

Lowest detectable limits for FMA using MALDI-TOF mass spectrometer provided by Bioscreen.

Genera/Group	**Lowest Detectable Limit**
Bacteroides	$5x10^8$
Bifidobacterium	$5x10^5$
Clostridium	$5x10^8$
Citrobacter	$5x10^5$
E. coli coliform	$7x10^6$
Enterobacter	$5x10^5$
Enterococcus	$5x10^5$
Eubacterium	$1x10^8$
Fungi/yeast	$1x10^4$
Klebsiella	$5x10^5$
Lactobacillus	$5x10^5$
Non-E. coli coliforms	$5x10^5$
Other Aerobes	$5x10^5$
Other Anaerobes	$1x10^8$
Porphyromanas	$5x10^8$
Prevotella	$5x10^9$
Serratia	$5x10^5$
Staphylococcus	$5x10^6$
Streptococcus	$3x10^5$